MANAGEMENT OF PLANT-PARASITIC NEMATODES AND SOIL HEALTH USING OIL RADISH (*RAPHANUS SATIVUS*) AND BROWN MUSTARD (*BRASSICA JUNCEA*) COVER CROPS

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ABSTRACT

This dissertation is composed of five chapters. Chapter one reviews factors that affect efficacy of biofumigation on management of plant-parasitic nematodes, focussing on *Meloidogyne* spp. in cropping systems and highlights two challenges. First, a number of literatures highlighted that susceptibility of biofumigant crops to target plant-parasitic nematodes could be an important management challenge and second, biofumigation being biocidal could have non-target impacts on free-living nematodes as bioindicators of soil health. Chapter two takes an alternative approach and elucidates the challenge of susceptibility as an opportunity to capitalize on as a trap crop arguing that using a good brassica host to a target nematode would be more effective as a conventional trap crop than using a poor host. When comparing trap cropping effects of 'Sodbuster' oil radish (OR; *Raphanus sativus*) as a poor host and 'Caliente 199' brown mustard (MS; *Brassica juncea*) as a good host against *Meloidogyne* spp. and *R. Reniformis*, MS showed potential as a trap crop depending on how long the trap crop was grown. MS suppressed soil population densities of *Meloidogyne* spp. in first and second trials by 60 and 50%, respectively where the cover crop was terminated within 42 days after planting (DAP; $P \leq 0.05$) but not in third trial when terminated 49 DAP. However, population densities of *R. reniformis* were not suppressed by MS in the first two trials where it was terminated 42 DAP but were suppressed by 61% ($P \leq 0.05$) in the third trial when the MS was terminated 49 DAP. Chapter three confirmed with previous studies that tissue maceration is necessary to activate myrosinase-glucosinolate system in brassica tissues to release bioactive isothiocyanates (ITC), soil tillage is required for the tissues to be in contact with the nematodes, and covering black plastic mulch is important to retain ITC from volatilization loss, together to maximize biofumigation effect on *Meloidogyne* spp. and *R. reniformis*. In three field trials conducted using OR and MS, soil

populations of *Meloidogyne* spp. were suppressed by OR or MS if the biofumigant crops were macerated (M), tilled (T) into the soil and covered with black plastic (BP) in all the trials, and reduced zucchini root galls in Trials I and II. However, suppression of *Meloidogyne* spp. was stronger when using MS than OR in the MTBP treatment. Regardless, MTBP suppressed *R. reniformis* in Trial I but not in Trials II and III. None-the-less, the trend appeared that MTBP reduced *R. reniformis* by 33.9 and 54.9% in Trials II and III, respectively. MTBP also stimulated zucchini growth in Trials I and III, but not in II. Chapter four investigated whether biofumigation could have non-target impacts on free-living nematode as indicators of soil health. Both OR and MS did not compromise soil health but instead OR enhanced nutrient enrichment throughout zucchini growth while MS did transiently for up to 1 month after biofumigation. Terminating both OR and MS by MTBP enhanced soil health indicators but suppressive to plant-parasitic nematodes. As indicators of biofumigation, Myr activity (based on soil glucose analysis) and soil sulfate analysis were conducted to establish relationships with soil health indicators and other response variables. Myrosinase activity had a strong positive relationship with soil health indicators when toluene (methylbenzene) was added in soil samples to arrest microbial degradation of glucose. However, sulfate was stable in the soil without toluene and even had a stronger positive relationship with the soil health indicators, thus a good indicator of biofumigation in the field. Chapter five concludes the findings and provide recommendations and future directions to enhance biofumigation effects of brassicaceous cover crops against plant-parasitic nematodes.

Key words: biofumigation, green manure, myrosianse activity, reniform nematode, root-knot nematodes, sulfate, termination methods, trap crop.

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CHAPTER 1

USE OF BRASSICACEOUS COVER CROPS AS CONVENTIONAL TRAP AND BIOFUMIGANT CROPS FOR MANAGING *MELOIDOGYNE* SPP. AND SOIL HEALTH: A REVIEW

Abstract

Although biofumigation employing brassica cover crops has been extensively researched, suppression of plant-parasitic nematodes, in particular *Meloidogyne* spp., continues to be inconsistent in the fields. Most brassicaceous cover crops are susceptible to *Meloidogyne* spp., highlighting one of the major challenges of biofumigation. Nevertheless, the brassica biofumigant host may stimulate hatch of nematode eggs in the soil, and second stage juveniles of *Meloidogyne* spp. are most vulnerable to biofumigation. While emphasizing the termination methods used to maximize the biofumigation effect, this review elucidates the challenge of biofumigant crops susceptible to *Meloidogyne* spp. and suggests susceptibility can be an opportunity to capitalize on as a trap crop. Any *Meloidogyne* spp. that completes a life cycle and hatches would be subjected to biofumigation. From a plethora of published research and a myriad of information available on biofumigation, this article highlighted a systematic approach to manage *Meloidogyne* spp. in the field through integration of conventional trap cropping and biofumigation, while enhancing the soil health by cover cropping with brassica crops.

Key words: biofumigation, degree-day, green manure, heat unit, management, root-knot nematodes.

1.1. Introduction

More than 4,100 species of plant-parasitic nematodes are known, posing an important threat to global food security (Decraemer and Hunt, 2006). Crop losses inflicted by plant-parasitic nematodes are estimated at $125 billion annually worldwide with at least $10 billion in the United States alone (Chitwood, 2003; Decraemer and Hunt, 2006). Those nematodes in the genus Meloidogyne are ranked among the most serious plant-parasitic nematodes based on their economic and scientific importance (Jones et al., 2013). To date, 98 species of Meloidogyne have been described (Ntalli and Caboni, 2017). Meloidogyne spp. are biotrophic, polyphagous infecting a wide range of crops, most of which have low economic damage thresholds (Sasser and Freckman, 1987).

Since the onset of Green Revolution, soil fumigation has been an effective but non-discriminative approach to manage soil-borne pests and pathogens, including plant-parasitic nematodes, in agroecosystems. However, fumigants such as methyl bromide have been banned or their use is being stringently restricted as with 1, 3-dichloropropene (1, 3-D) (Hillocks, 2012). In California, application of 1, 3-D is restricted to 372.1 kg a.i/ha per year and its use is prohibited within 30.5 m of any occupied structure (California Department of Pesticide Regulation, 2017).

Scientists in search of safer and environmentally friendly alternatives to manage plant-parasitic nematodes have looked into cover crops with allelopathic compounds. Monocrotaline from sunn hemp, *Crotolaria juncea* (Wang et al., 2002), α-tertienyl from French marigold, *Targetes* spp. (Hooks et al., 2010), dhurrin from sorghum-sudangrass, *Sorghum* × *drummondii* (Widmer and Abawi, 2000), L-dopa from velvet bean, *Mucuna pruriens* (Zasada et al., 2006)

and glucosinolates (GL) from members of Brassicaceae (Halbrendt, 1996; Gimsing and Kirkegaard, 2006; Ploeg, 2008; Zasada et al., 2009; Dandurand et al., 2017) have been identified. This review focuses on integrating trap cropping and biofumigation to maximize nematode suppressive effects and improve soil heath using brassica cover crops.

Ploeg (2008) and Fourie et al. (2016) presented rather comprehensive reviews of *Brassica* spp. efficacy as biofumigants and identified the host status of a wide range of *Brassica* spp. and cultivars to *Meloidogyne* spp. Most of these reviews considered that host susceptibility to the target nematodes is in conflict with biofumigation efficacy. We exploit this gap with an alternative position where host susceptibility in fact would enhance biofumigation efficacy with a sophisticated time-sensitive process of stimulating egg hatching with subsequent targeted release of isothiocyanates (ITC) at the most vulnerable life stage. In addition, this article highlights the methods of terminating biofumigant cover crops to achieve maximum GL hydrolysis and release of ITC to improve biofumigant effect. Furthermore, albeit the ultimate goal of growing a cover crop prior to cash crop planting is to improve soil health, an underlining concern is that the biocidal effect of ITC could compromise soil health. An ideal impact of cover crops would be a combined effect of improving soil health while managing plant-parasitic nematodes via conventional trap cropping and biofumigation to construct a beneficial sustainable farming system.

1.2. Biofumigation

The original definition of biofumigation coined by Kirkegaard et al. (1993) refers to the use of GL-derived ITC from brassica cover crops to suppress soil-borne pests and pathogens.

Glucosinolates or *β*-d-thioglucose thioglycosides are naturally occurring secondary metabolites biosynthesized by members of Brassicaceae, which are stored in vacuole of sulfur-rich S-cell (Fig. 1.1A). These compounds are spatially separated from myrosinase (Myr) enzyme or *β*-thioglucosidase (EC3.2.1.147) which is stored as myrosin grain in the vacuole of a particular idioblast known as myrosin cell (Fig. 1.1B) (Höglund et al., 1991; van Dam et al., 2009; Li et al., 2014). To date, at least 200 GL have been identified of which more than 80 % occur in Brassicaceae (Fahey et al., 2001; van Dam et al., 2009; Agerbirk and Olsen, 2012; Bischoff, 2016). Each GL is constituted of a *β*-thioglucose moiety, a sulphonated oxime moiety, and a thiohydroximate-O-sulfonate moiety (Fig. 1.2) (Fenwick et al., 1983). Glucosinolates can be categorized as aliphatic, aromatic or indole if the amino acid side chain denoted as R (Fig. 1.2) is methionine, phenylalanine or tryptophan, respectively (Fig. 1.2) (Velasco et al., 2008). Only upon tissue maceration, does Myr come into contact with GL and chemically hydrolyze the thioglucoside linkage (carbon-sulfur bond), yielding *D*-glucose and thiohydroxymate-O-sulfonate (aglycone), an unstable intermediate. The aglycone undergoes a non-enzymatic rearrangement in a spontaneous fashion to form volatile products including ITC, nitriles, and thiocyanates, and non-volatile products including sulfate and sulfur (Cole, 1976; Fenwick et al., 1983).

Documentation of the suppressive effects of Brassicaceae can be dated back to 1925 when population densities of nematodes exposed to mustard residues were reduced (Morgan, 1925). This observation was followed by research on residues and breakdown products of brassica crops that acted as bionematicides on *Heterodera schachtii* (Smedley, 1939). However, the mode of action against nematodes by brassica crops was not known until 1993 (Kirkegaard et al., 1993). Brassica cover crops possess biocidal attributes that include bactericidal (Dufour et al., 2015),

fungicidal (Aparna and Girija, 2018), herbicidal (Brown and Morra, 1995; Lefebvre et al., 2018), insecticidal (Sukovata et al., 2015), and nematicidal properties (Ploeg, 2008; Lazzeri et al., 2009; Zasada et al., 2009; Lord et al., 2011; Rahman et al., 2011; Avato et al., 2013; Ngala et al., 2015; Fourie et al., 2016; Mashela et al., 2017). All GL in brassica plants were enzymatically converted to ITC via hydrolysis by Myr (Kirkegaard et al., 1993). Isothiocyanates have biocidal properties (Kirkegaard and Sarwar, 1998) similar to the synthetic nematicidal fumigant, methyl isothiocyanate released from metam sodium and dazomet (Matthiessen and Kirkegaard, 2006). Different species, cultivars or even tissues of brassica crops produce different ITC with variable concentrations and different nematode toxicities (Table 1.1).

Besides using brassica cover crop residues, defatted seed meals generated from certain brassica cover crops have been used for biofumigation (Wu et al., 2011; Mocali et al., 2015; Dandurand et al., 2017). In addition, a specific biotechnological formulation has been developed from defatted seed meal that resulted in a liquid product appropriate for drip irrigation (Lazzeri et al., 2008) to suppress *M. incognita* (De Nicola et al., 2013). Furthermore, defatted seed meal extract has been formulated into a powdered form with a longer shelf life compared to the defatted seed meal (Popova and Morra, 2017). Dandurand et al. (2017) reported that half as much *B. juncea* seed meal extract was required to achieve LC_{90} for hatch of either *Globodera ellingtonae* or *G. pallida* eggs compared to that of a defatted seed meal formulation.

Despite a plethora of researches conducted on the nematode suppressive effect of biofumigation, results have been inconsistent (Lazzeri et al., 2003; Stirling and Stirling, 2003; Matthiessen and Kirkegaard, 2006), stimulating a multitude of studies aimed at optimizing biofumigation efficacy (Kirkegaard and Matthiessen, 2004). Efficacy of biofumigation on soil-borne pests and pathogens is depending on various factors including 1) the species or cultivar of

the biofumigant cover crop, 2) the agronomic practices such as application of sulfur (S) and nitrogen (N) fertilizers, 3) edaphic factors such as soil physical, chemical and biological properties, 4) biofumigation methods including tillage, tissue maceration, and tarping, and 5) sensitivity of life stages of *Meloidogyne* spp. to ITC. While optimizing conditions that favor effective biofumigation, the challenges of biofumigation often overlooked are that 1) most biofumigant crops are susceptible to *Meloidogyne* spp. (Table 1.2), and 2) biofumigation could compromise soil health by affecting non-target organisms. By understanding these factors and challenges, we hope to refine biofumigation protocols that suppress plant-parasitic nematodes while managing soil health.

1.3. Factors Affecting Biofumigation

Biofumigant cover crops

Members of Brassicaceae constitute some 350 genera and 3,500 species (Rosa et al., 1997; Abu-Ghannam and Jaswal, 2015). Brassica cover crops commonly used for biofumigation include brown mustard (*Brassica juncea*), yellow/white mustard (*Sinapis alba; Brassica hirta*), rape seed (*Brassica napus*), field mustard (*Brassica rapa* var. *rapa*) and oil radish (*Raphanus sativus*) (Kirkegaard and Sarwar, 1998). The type and concentration of GL vary among species, cultivars, and even tissues within a cultivar (Bellostas et al., 2004; Gimsing and Kirkegaard, 2006). The carboxyl group (R-Group) determines the type of GL. For example, if the R-Group is propenyl, the compound is referred to as propenyl GL with common name sinigrin. Sinigrin is the dominant GL in *B. juncea* and *B. nigra* (Kirkegaard and Sarwar, 1998). Vervoort et al. (2014) determined total GL and sinigrin levels of *B. juncea* ‘Terrafit’, ‘Terratop’, ‘Terraplus’ and

'ISCI99' to be different not only among cultivars but also among tissues. 'ISCI99' generated more biomass and accumulated higher concentrations of both total GL and sinigrin in roots than in foliage, compared to the other cultivars tested (Vervoort et al., 2014). In addition, in intact plant tissues specifier proteins could interact with Myr to determine GL hydrolysis products (Fig. 1.2). Presence of specifier proteins favors thiocyanate or nitrile formation than ITC (Kissen et al., 2012; Hanschen et al., 2015). Furthermore, growth stage of the plant affects the concentration of GL (Bellostas et al., 2004, 2007). Whereas concentration of GL in roots and stems decreases gradually as the plant develops, it increases in leaves and reproductive organs of *B. juncea* (Bellostas et al., 2007). Growing season also affects concentration of GL in brassica cover crops (Ngala et al., 2015). The highest GL production was achieved in summer followed by spring, indicative of higher growing degree-days and corresponding biomass production (Reddy, 2011; Ngala et al., 2015).

Agronomic practices

Application of sulfur (S) and nitrogen (N) fertilizers is important because these elements are integral components of GL (Fig. 1.2) (Falk et al., 2007; Groenbaek et al., 2016). Low N and high S fertilizer application enhanced aliphatic GL in *B. rapa* (Chen et al., 2006). Li et al. (2007) noted that whereas total GL concentration was not affected by fertilizer inputs, individual GL concentration was affected by S or N supply. Nitrogen containing tryptophan-derived indole GL was directly proportional to N supply whereas S containing methionine-derived aromatic GL were inversely proportional to N supply (Li et al., 2007). Application of N-containing fungicide, metconazole increased total GL concentration in *B. juncea* and *R. sativus* (Ngala et al., 2015).

Biofumigation methods

Although incorporation of brassica tissue into the soil is the conventional method of biofumigation (Mazzola et al., 2007; Meyer et al., 2011; Ngala et al., 2015), crop rotation or intercropping with brassica crops also release negligible amount of ITC into the rhizosphere and had shown potential to suppress soil-borne pathogens (van Dam et al., 2009). This could be due to release of ITC through leaf washings, root exudates or mechanical damage by herbivorous pests. For example, cabbage root fly larvae (*Delia radicum*) feeding on *R. sativus* roots released ITC into rhizosphere and was claimed to be toxic to *G. pallida* encysted eggs (Ngala et al., 2015). Therefore a broader range of biofumigation methods are outlined in Fig. 1.3 that include soil incorporation or covering the soil with different mulch with or without tissue maceration, watered or not watered, or simply apply through drip irrigation. In addition, intercropping or crop rotation between biofumigant crops and cash crops are also taking into consideration for the reasons stated above (van Dam et al., 2009; Ngala et al., 2015).

Liquid formulation was prepared from defatted seed meal delivered through drip irrigation (De Nicola et al., 2013). Biofumigation employing liquid formation was demonstrated to be suppressive to *M. incognita*, the suppressive effect was strongly correlated with the dose and release of allyl ITC, and can reach nematodes in deeper soil profile following the coverage of the liquid. Thus, liquid formulation of defatted seed meal is of advantage over using seed meal extract or powdered form (Popova and Morra, 2017). Efficacy of biofumigation using solid or powdered form was dependent on the depth of soil incorporation to be exposed to the nematodes.

However, as the knowledge on the mechanism of ITC production becomes apparent, the conventional method of biofumigation has shifted to include tissue maceration, irrigation and/or tarping with impermeable film. The fact that GL and Myr are spatially separated in intact plant

cells (Fig. 1.1), tissue maceration would enhance GL hydrolysis thus maximize ITC production and biofumigation effect (Morra and Kirkegaard, 2002; Matthiessen et al., 2004). Effective biofumigation occurs when hydrolysis of GL generates more than 100 nmol of ITC/g soil (Gimsing and Kirkegaard, 2009). In addition, with the knowledge that water mediates GL hydrolysis, it is beneficial to provide irrigation after the tissue maceration and soil incorporation to maximize hydrolysis while leaching the ITC into deeper soil profile to be in contact with the nematodes. It has been reported that irrigation with 34 mm in a field after pulverizing *B. juncea* tissues produced 100 nmol/g soil of propenyl ITC (Matthiessen et al., 2004), with a biofumigation effect equivalent to the 200 nmol methyl ITC/g soil from metam sodium (Matthiessen and Kirkegaard, 2006). Furthermore, with the understanding that aliphatic ITC are volatile (Ntalli and Caboni, 2017), maximum biofumigation effectiveness requires soil sealing with plastic film immediately after tissue maceration and soil incorporation (Kirkegaard and Matthiessen, 2004). Use of black plastic mulch is more advantageous than clear solarization mulch (Ohtsuru et al., 1973) because of its low solar radiation transmittance and would be less destructive to Myr and beneficial soil microorganisms. Stapleton and Duncan (1998) also recommended to tarp the soil for no more than 7 days to avoid anaerobic soil disinfestation (Blok et al., 2000; Ueki et al., 2018). This is because under anaerobic soil conditions, redox potential decline and generate Fe^{2+} (Momma et al., 2013) as well as organic acids that would interfere with ITC production.

Soil physical properties

Soil physical properties that would alter biofumigation effects include soil water content, texture and temperature. Although soil moisture is needed for hydrolysis of GL to occur, it can

also affect the half-life of GL. For example, benzyl GL only had a half-life of 6.8-15.5 h when the soil: water content ratio was 1:1 but its half-life increased to 17.5-195 h if the soil moisture was reduced to 8-11.6 % (Gimsing et al., 2006). In general, biofumigation is improved when soil moisture is maintained at optimum levels (Matthiessen et al., 2004). However, too much water can leach GL from the biologically active soil profile. This is because GL is adsorbed weakly to soil particles (Gimsing and Kirkegaard, 2009; Omirou et al., 2013). On the other hand, soil texture plays an important role in the degradation of GL. In the topsoil, GL degraded rapidly in clay soil than in sandy soil. However, in the clay subsoil, the degradation rate of GL would be reduced due to lack of biological activities to an extent of no degradation in sandy subsoil (Gimsing et al., 2006).

In terms of soil temperature, volatilization of ITC would increase with increasing temperature, and might lead to loss of ITC if it is not contained in the soil (Price et al., 2005). Temperature is especially affecting short-chained aliphatic GL in brassica crops such as *B. napus* (Mojtahedi et al., 1993; Charron and Sams, 2004). The implication is that control of citrus nematode, *Tylenchulus semipenetrans*, by metam sodium fumigation was increased by 30 % at 20°C compared to 10°C (Klose et al., 2008).

Soil chemical properties

Soil pH, iron and organic matter are important chemical properties that influence ITC production (Uda et al., 1986). These chemical properties determine non-enzymatic rearrangement of the intermediate product of GL hydrolysis, aglycone, to form ITC, nitriles or thiocyanates (Fig. 1.2). Lower pH favors nitrile production whereas higher pH favors ITC production (Gil and MacLeod, 1980; Borek et al., 1994). At pH < 6, aglycone undergoes proton

(H^+) dependent desulfuration to yield nitrile and elemental sulfur (Uda et al., 1986; Borek et al., 1995). In contrast, at pH ≥ 6, aglycone experiences a concerted loss of sulfate ($SO4^{2-}$) independent of proton (H^+) in Lossen rearrangement and produces ITC (Uda et al., 1986).

In addition, at low pH, humic acid and goethite, an iron-containing mineral, would adsorb GL and result in poor biofumigation (Gimsing et al., 2007). Presence of ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions would divert the hydrolysis process of GL to produce nitrile (Youngs and Perlin, 1976; Hanschen et al., 2015). To further proving the effect of Fe^{2+}, Hanschen et al. (2015) autoclaved a soil to increase Fe^{2+} content and observed an antagonistic effect against biofumigation. Moreover, presence of Fe^{3+} can nearly terminate both allyl nitrile and allyl ITC production (Hasapis and MacLeod, 1982; Uda et al., 1986; Borek et al., 1994).

In terms of soil organic matter, hydrophobic ITC are adsorbed to soil organic matter, thus reducing their biofumigation activities (Brown and Morra 1997; Matthiessen and Shackleton, 2005; Gimsing and Kirkegaard, 2009). Sorption of ITC to organic matter increase with their non-polar nature (Gimsing et al., 2009). Price et al. (2005) reported that incorporation of *B. juncea* tissue in sandy soil with less organic matter had lower ITC in the air above it than clay soil with high organic matter content. Matthiessen and Shackleton (2005) also noted that higher soil organic matter at low temperature significantly reduced ITC volatility and thus resulted in low biofumigation efficacy.

Soil microbiota

Degradation of benzyl GL was arrested in autoclaved soil but not in soils treated with γ-irradiation or azide that did not inactivate the Myr enzymes (Gimsing et al., 2006). Thus, Gimsing et al. (2006) concluded that only Myr was responsible for GL degradation. In contrast,

Hanschen et al. (2015) observed slower degradation of GL in autoclaved soil, and elimination of soil microbes resulted in the formation of nitriles instead of ITC when the GL incubation was conducted in sterile soil conditions. Although Gimsing and Kirkegaard (2009) argued that microbial degradation of GL in soil without Myr did not produce ITC, they did report that the efficacy of biofumigation was profoundly affected by soil microorganisms. However, several reports revealed that some soil microorganisms could produce Myr when GL were present in the soil (Sakorn et al., 1999, 2002; Omirou et al., 2013). For example, *Aspergillus niger,* an ubiquitous soil-borne facultative parasite (Ohtsuru et al., 1973), *Aspergillus* sp. NR-4201 (Sakorn et al., 1999), *Enterobacter cloacae,* a bacterial antagonist of *Fusarium oxysporum* and *Pythium* spp. (Tani et al., 1974) all produced Myr when GL was incorporated into the soil. In addition, the soil-borne bacterium *Citrobacter* WYE1 was found to possess an inducible *β*-glucosidase capable of transforming GL into ITC (Albaser et al., 2016). In any case, cultural practices that enhance these soil microorganisms could enhance conversion of GL to ITC (Sakorn et al., 1999). Thus, Myr production of ITC is enhanced by the soil microbiota.

Sensitivity of Meloidogyne stages to ITC

While sensitivity to ITC varies between nematode species (Zasada and Ferris, 2003), various developmental stages of *Meloidogyne* spp. react differently to ITC. Mojtahedi et al. (1993) and Ploeg (2008) highlighted that J2s of *M. chitwoodi* were more vulnerable to biofumigation than eggs. Similarly, J2s of *M. incognita* were more sensitive to defatted seed meals of brassicas compared to mixed stages of *Pratylenchus penetrans* (Zasada et al., 2009). Furthermore, Gilreath and Santos (2004) reported that metam sodium was more effective against the target pest when the organisms were actively respiring.

1.4. Challenges in Biofumigation

Susceptible host plant

Most brassica cover crops used in biofumigation are susceptible to *Meloidogyne* spp., posing a risk to increase population densities of plant-parasitic nematodes (Monfort et al., 2007; Edwards and Ploeg, 2014; Fourie et al., 2016). Whereas most cultivars of *B. juncea* and *B. rapa* were reported as good hosts of *Meloidogyne* spp., *Eruca sativa* 'Nemat' and *R. sativus* 'Boss' including 'TerraNova' were ranked among the poorest hosts (Stirling and Stirling, 2003; Monfort et al., 2007; Edwards and Ploeg, 2014). Host status of a list of brassica crop cultivars to *Meloidogyne* spp. being studied are cited in Table 1.2.

In attempts to address undesired nematode reproduction on brassica cover crops, a number of studies have recommended to use poor or non-host cultivars of *Meloidogyne* spp. (Edwards and Ploeg, 2014; Ntalli and Caboni, 2017). Although use of poor host can avoid *Meloidogyne* spp. reproduction, it will not be able to stimulate egg hatch to J2 stage, which is more vulnerable to biofumigation (Mojtahedi et al., 1993; Ploeg, 2008). Another school of thought is to grow nematode susceptible brassica cover crops during winter to limit nematode development and delay egg production (Stirling and Stirling, 2003). However, this approach does not apply in tropical climatic regions where temperatures remain above the nematode development thresholds all year round.

Negative impact on soil health

Soil health is the capacity of a soil to function as a vital living system, within ecosystem and land-use boundaries, maintain or enhance water and air quality, and promote plant and animal productivity and health (Doran and Zeiss, 2000). Isothiocyanates being biocidal are likely to adversely impact non-target microorganisms and can compromise soil health (Cao et al., 2004). Bending and Lincoln (2000) reported that communities and activity of soil nitrifying bacteria were inhibited by GL hydrolysis products. However, Vervoort et al. (2014) noted that observed changes in nematode communities after the practice of biofumigation were not due to the ITC allelopathic effect but rather due to the intense mechanical disturbance during soil incorporation. While Gruver et al. (2010) concluded that radishes stimulated bacterial decomposition, Valdes et al. (2012) reported that amendment of *S. alba* 'Zlata' decreased the abundance of plant-parasitic nematodes and increased the beneficial nematode community. Similarly, Ferris et al. (2001) reported that *S. alba* amended plots increased nematode enrichment index and decreased the channel index. In fact, even when oil radish residues were not soil incorporated, no-till 'Sodbuster' oil radish cover cropping following tissue maceration also increased the numbers of bacterivorous nematodes (Marquez, 2017), indicating a soil food web with a decomposition pathway dominated by bacteria.

On the other hand, Henderson et al. (2009) observed biofumigation with green manure and seed meal of *B. carinata* negatively affected the ability of entomopathogenic nematodes, *Steinernema* spp., *S. feltiae* and *S. riobrave,* to infect Colorado potato beetle, *Leptinoarsa decemlineata*. In addition, Ramirez et al. (2009) reported that mustard biofumigants reduced foraging ability of *Steinernema* and *Heterorhabditis* spp. Furthermore, maceration of *R. sativus* 'Sodbuster' foliage in a no-till cover cropping system reduced infectivity of mealworm (*Tenebrio molitor*) larvae by *Heterorhabditis* (Marquez, 2017). Thus, biofumigation using brassica cover

crops can have negative impacts on entomopathogenic nematodes.

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Tables

Table 1.1. Nematode suppressive effects of different biofumigant crop species affected by their cultivars/accessions, form of application, amendment rates, glucosinolate concentration, and target nematodes.

Biofumigant crop				Total (ITC-generating) GL		Nematode		
Species	Cultivar/ Accession	Form[1]	Amendment rate[2]	μmol g^{-1} dw[3]	nmol g^{-1} soil[4]	Species	Suppression[5]	References
B. carinata	Acc. 94044	GM	2.0 %	21.7 (21.5)	86.8 (85.3)	*Pratylenchus neglectus*	32.6 %	Potter et al., 1998
	BRK-147A	GM	na	30.6	135.4	na	na	Bellostas et al., 2004
	BRK-147A	S	na	116.0	na	na	na	Bellostas et al., 2007
	ISCI7	SM	2.5 t/ha	163.4 (160.1)	na	*Meloidogyne chitwoodi*	>80.0 %	Henderson et al., 2009
	ISCI7	SM	3.0 t/ha	150.7 (147.7)	na	*M. incognita*	<RGI	Lazzeri et al., 2009
	na	LF	6.0 % (v/v)	90.0	na	*M. incognita*	81.0 %	De Nicola et al., 2013
B. hirta	Martegena	GM	na	73.1	na	*M. javanica*, *T. semipenetrans*	na	Zasada & Ferris, 2003
B. juncea	Acc. 99Y11	GM	2.0 %	20.4	81.6	*P. neglectus*	40.9 %	Potter et al., 1998
	Caliente 99	GM	230.0*	62.5 (49.2)	na	*Globodera pallida*	effective	Ngala et al., 2015

Caliente 61	GM	0.1 t/ha	49.1 (36.3)		*M. incognita*	no effect	Rudolph et al., 2015
Cutlass	GM	na	11.7	135.4	na	na	Bellostas et al., 2004
ISCI99	GM	9.9 t/ha	29.0 (25.0)	100.5 (91.4)	*Trichodorus, Tylenchorynchus*	no effect	Vervoort et al., 2014
	GM	1.1 t/ha	72.1 (58.4)	na	*M. incognita*	no effect	Rudolph et al., 2015
JR049	GM	5.6 t/ha	6.7 (4.9)	44.6 (40.4)	na	na	Gimsing & Kirkegaard, 2006
Nemfix	GM	10.3 t/ha	22.5 (20.2)	169.9 (161.6)	*M. javanica*	9.0 fold	Rahman & Somers, 2005; Gimsing & Kirkegaard, 2006
Nemfix	SM	2.0 t/ha	na	na	*M. javanica*	9.0 fold	Rahman & Somers, 2005
Pacific Gold	SM	1.2 t/ha	153.2 (152.0)	na	*M. incognita, P. penetrans*	>90.0 %	Zasada et al., 2009
	GM	1.2 t/ha	57.7 (45.9)	na	*M. incognita*	no effect	Rudolph et al., 2015
Pacific Gold	SM	>2.2 t/ha		na	*G. pallida*	100.0 %	Zasada et al., 2009; Dandurand et al., 2017
Pacific Gold	SM	>4.5 t/ha			*G. ellingtonae*	>92.1 %	Dandurand et al., 2017
Pacific Gold	S	na	61.0	na	na	na	Bellostas et al.,

								2007
	Pacific Gold	SME	1.1 t/ha	278.0 (278.0)		*G. ellingtonae*	100.0 %	Dandurand et al., 2017
	Terrafit	GM	6.9 t/ha	22.2 (19.3)	61.1 (55.8)	*Trichodorus, Tylenchorynchus*	no effect	Vervoort et al., 2014
	Terraplus	GM	7.5 t/ha	20.1 (15.4)	63.4 (54.5)	*Trichodorus, Tylenchorynchus*	no effect	Vervoort et al., 2014
	Terratop	GM	8.4 t/ha	16.7 (13.1)	61.8 (52.5)	*Trichodorus, Tylenchorynchus*	no effect	Vervoort et al., 2014
B. napus	BQ Mulch	GM	7.0 t/ha	25.7	164.5 (91.9)	na	na	Gimsing & Kirkegaard, 2006
	Dunkeld Acc. 94713	GM	2.0 %	7.5 (6.8)	28.8 (24.0)	*Pratylenchus neglectus*	44.5 %	Potter et al., 1998
	Dwarf Essex	SM	5.0 t/ha	41.9 (35.6)	na	*M. incognita*	90.0 %	Zasada et al., 2009
	Dwarf Essex	SM	50.0 t/ha	41.9 (35.6)	na	*P. penetrans*	90.0 %	Zasada et al., 2009
	MaximaPlus	GM	7.7 t/ha	16.6 (9.0)	78.1 (21.3)	na	na	Gimsing & Kirkegaard, 2006
	Sunrise	SM	15.0 t/ha	14.8 (3.0)	na	*M. incognita, P. penetrans*	no effect	Zasada et al., 2009
B. nigra	Acc. 95067	GM	2.0 %	16.4 (16.4)	65.4 (65.4)	*P. neglectus*	28.1 %	Potter et al., 1998
	Giebra	GM	na	22.5	647.6	na	na	Bellostas et al., 2004

	Giebra	S	na	193.0	na	na	na	Bellostas et al., 2007
B. oxyrrhina	Acc. 95060	GM	2.0 %	34.0 (33.4)	136.1 (133.8)	*P. neglectus*	71.8 %	Potter et al., 1998
B. rapa	Harmoni	GM	na	3.6	15.7	na	na	Bellostas et al., 2004
	Harmoni	S		<30.0	na			Bellostas et al., 2007
	na	GM	2.0 %	3.2 (2.9)	12.9 (11.4)	*P. neglectus*	33.1 %	Potter et al., 1998
E. sativa	Nemat	GM	77.7 t/ha*	61 (36)	na	*G. pallida*	no effect	Ngala et al., 2015
R. sativus	Bento	GM	124.7 t/ha*	31.7 (27.8)	na	*G. pallida*	no effect	Ngala et al., 2015
S. alba	IdaGold	SM	20.0 t/ha	163.9 (156.8)	na	*P. penetrans*	65.0 %	Zasada et al., 2009
	IdaGold	SM	20.0 t/ha	163.9 (156.8)	na	*M. incognita*	90.0 %	Zasada et al., 2009
	IdaGold	SM	100.0 t/ha	163.9 (156.8)	na	*P. penetrans*	90.0 %	Zasada et al., 2009
	Zlata	GM	30.7 t/ha	na	na	*G. rostochiensis*	na	Valdes et al., 2012

[1]GM = green manure; S = intact seed; SM = defatted seed meal; SME = defatted seed meal extract in powder; LF = liquid formulation (prepared from defatted seed meal and liquid phase) mixed in water; na = data not available.

[2]Tissue amendment is based on dry weight unless indicated with * which is identified as fresh weight.

[3]Values outside of parentheses are average of total GL in dry shoot and root tissues of biofumigant crops, and values inside of parentheses are GL that only generate ITC.

[4]Values outside the parentheses are determined based on total GL in root and shoot per dry weight of soil (based on 10-cm soil depth, and 1.08 g cm^{-3} soil bulk densities). Values inside the parentheses are GL that only generate ITC.

[5]<RGI = reduced root gall index; effective = suppression was statistically significant.

Table 1.2. Host status of common biofumigant crops to major root-knot nematode, *Meloidogyne* species.

Biofumigant crop		*Meloidogyne* species			References
Species	Cultivar	*M. hapla*	*M. incognita*	*M. javanica*	
Brassica carinata	Bc007	Poor	Moderate	Poor	Edwards & Ploeg, 2014
Brassica juncea	ISCI99	Good	Good	Good	Edwards & Ploeg, 2014
	Nemfix	Good	Good	Good	Stirling & Stirling, 2003; Edwards & Ploeg, 2014
	Pacific Gold	Moderate/good	Good	Moderate	Monfort et al, 2007, Edwards & Ploeg, 2014
Brassica napus	Humus	Poor/moderate	Poor/moderate	Poor/moderate	Edwards & Ploeg, 2014
	Winfred	Poor	Moderate/good	Good	Edwards & Ploeg, 2014
Brassica rapa	Rondo	Good	Good	Good	Edwards & Ploeg, 2014
	Samson	Good	Good	Good	Edwards & Ploeg, 2014
Eruca sativa	Nemat	Poor	Poor	Poor	Curto et al., 2005; Melakeberhan et al., 2006; Edwards & Ploeg, 2014
		Poor	Poor	Poor	McLeod et al, 2001; Edwards & Ploeg,
Raphanus sativus	Adagio				2014
	Adios	Poor/moderate	Moderate/good	Poor/moderate	Edwards & Ploeg, 2014
		Poor	Poor	Poor	Curto et al., 2005; Edwards & Ploeg,
	Boss				2014
	Colonel	Good	Poor	Poor	Edwards & Ploeg, 2014

	Comet	Poor	Good	Poor	Edwards & Ploeg, 2014
	Defender	Poor	Poor	Poor	Edwards & Ploeg, 2014
	TerraNova	Good	Poor	Poor	Edwards & Ploeg, 2014
Sinapis alba	Abraham	Poor/moderate	Poor	Poor/moderate	Edwards & Ploeg, 2014
	Absolut	Poor	Moderate	Moderate	Edwards & Ploeg, 2014
	Accent	Poor	Poor	Poor	Edwards & Ploeg, 2014
	Achilles	Poor/moderate	Moderate/good	Moderate/good	Edwards & Ploeg, 2014
	Condor	Poor	Moderate/good	Poor	Edwards & Ploeg, 2014
	IdaGold	Good	Moderate/good	Moderate	Edwards & Ploeg, 2014
	Maxi	Moderate	Poor/moderate	Poor	Edwards & Ploeg, 2014
	Santa Fe	Poor/moderate	Moderate	Poor/moderate	Edwards & Ploeg, 2014

Figures

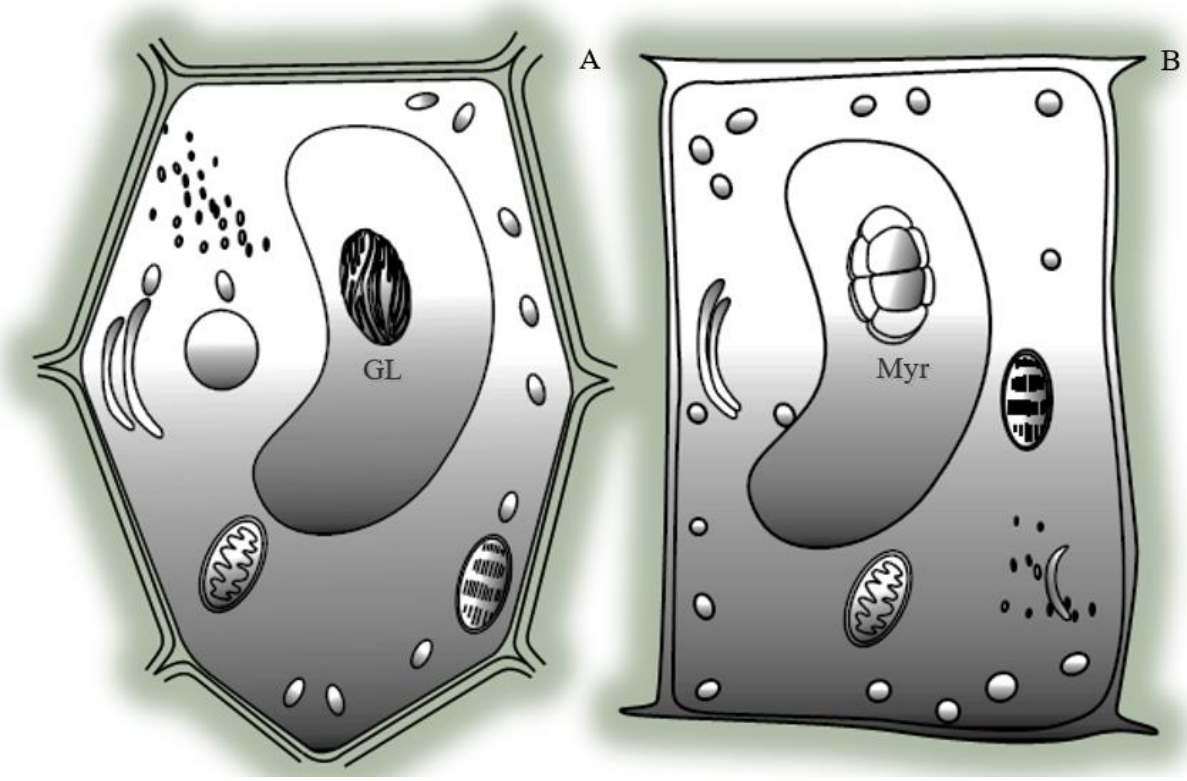

Fig. 1.1. A) Sulfur rich S-cell contains glucosinolate (GL), and B) myrosin cell contains myrosinase (Myr) (Li et al., 2014).

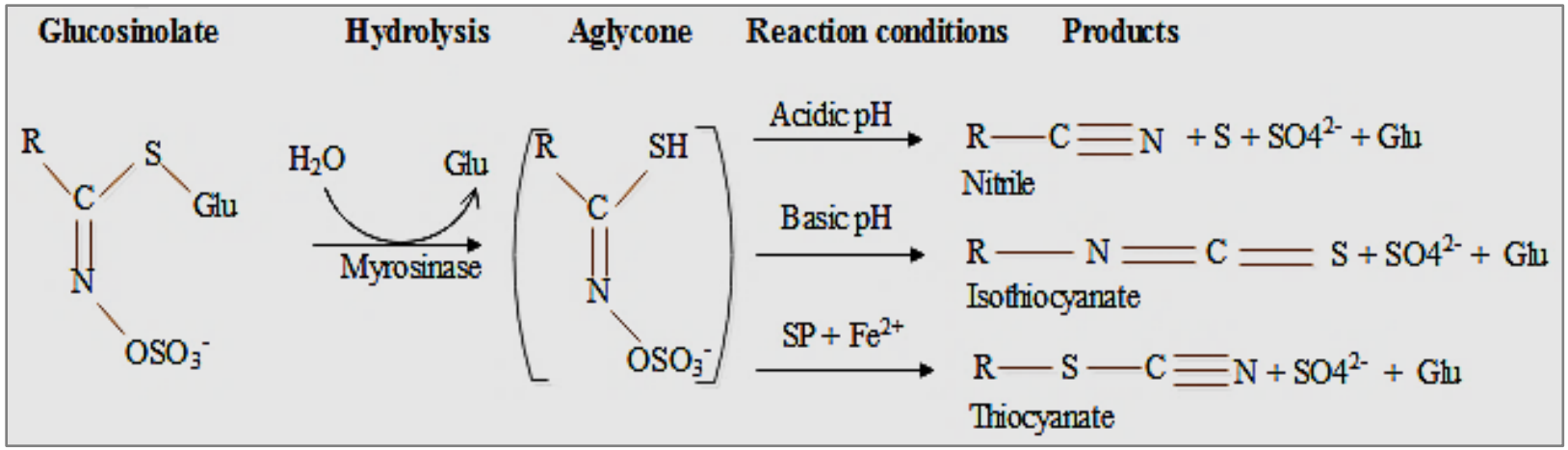

Fig. 1.2. Glucosinolate hydrolysis pathway modified from Kirkegaard (2009). Glu = glucose; R-N=C=S is isothiocyanate; R-C≡N; SP = specifier proteins; R-S-C≡N is an ionic thiocyanate.

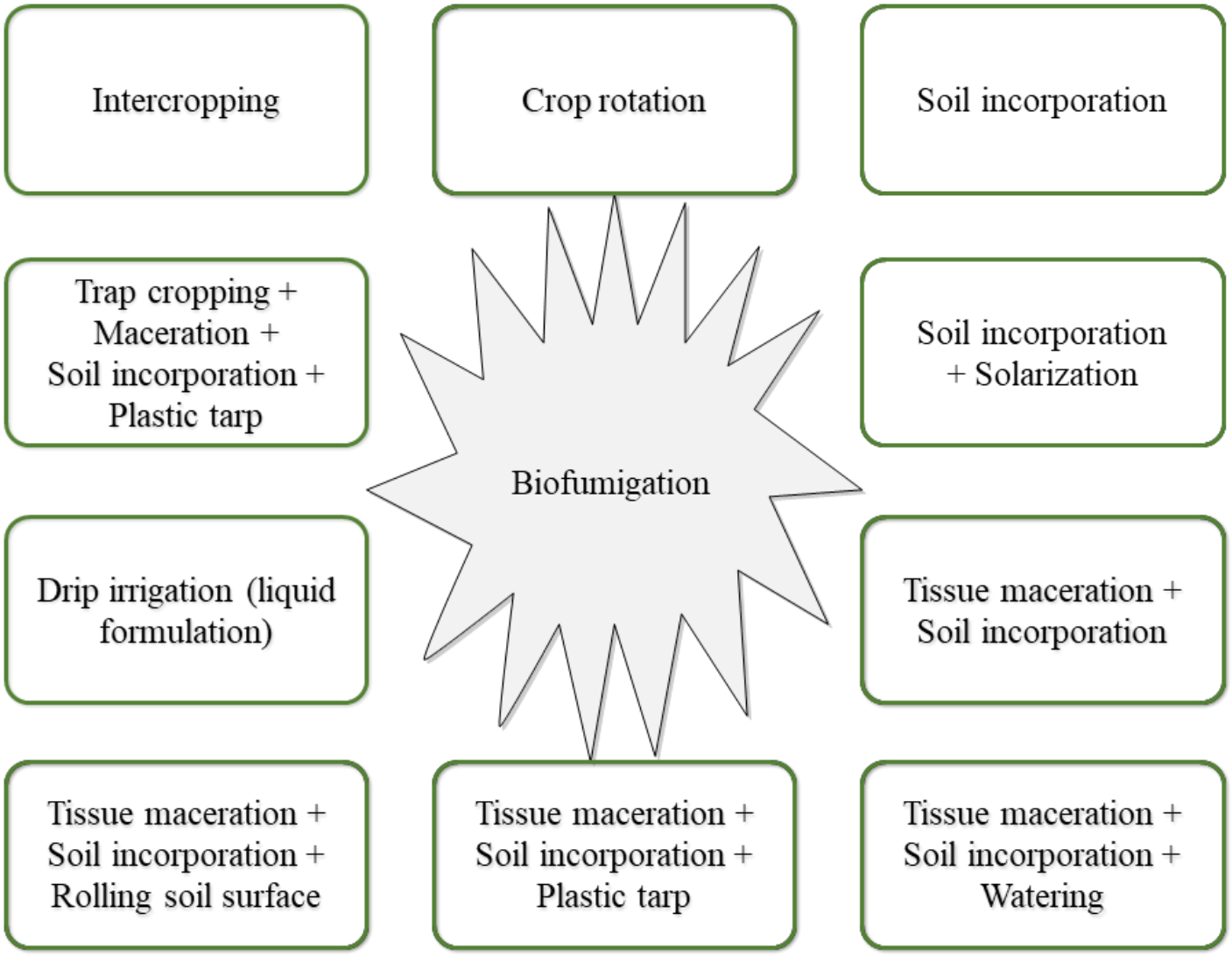

Fig. 1.3. Methods of biofumigation using brassica crops.

CHAPTER 2

CONVENTIONAL TRAP CROPPING POTENTIAL OF BRASSICA COVER CROPS FOR MANAGEMENT OF *MELOIDOGYNE* SPP. AND *ROTYLENCHULUS RENIFORMIS* IN THE TROPICS

Abstract

Brassicaceous cover crops provide a number of soil health improvement services but most species and cultivars are susceptible to root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes, posing an important management challenge for subsequent cash crops. This project explored conventional trap cropping potential of oil radish (*Raphanus sativus*) and brown mustard (*Brassica juncea*) against *Meloidogyne* spp. and *R. reniformis*. It was hypothesized that using a good brassica host as a trap crop of a targeted nematode would be more effective as the nematode trap crop than using a poor host. Greenhouse pot experiments determined that oil radish was relatively a poor host whereas brown mustard was a very good host of *M. incognita*. However, both brassica cover crops were very good hosts of *R. reniformis*. Stands of 'Sodbuster' oil radish were established in the field infested with the nematodes and terminated on 0, 14, 28, 42 and 56 days after planting (DAP) to determine the best termination time to achieve trap cropping effect. Although growing oil radish for 28 days did not suppress population densities of *Meloidogyne* spp., root-gall index on pumpkin (*Cucurbita moschata*), was reduced ($P \leq 0.05$) and pumpkin yield was increased by 74%. On the other hand, when 'Caliente 199' brown mustard was used as a trap crop, it suppressed soil population densities of *Meloidogyne* spp. in first and second trials by 60 and 50%, respectively

where the cover crop was terminated within 42 DAP ($P \leq 0.05$), but not in third trial when terminated 49 DAP. However, population densities of *R. reniformis* were not suppressed by brown mustard in the first two trials where brown mustard was terminated 42 DAP, but were suppressed by 61% ($P \leq 0.05$) in the third trial when the brown mustard was terminated 49 DAP. Thus, brown mustard showed potential as a trap crop against *Meloidodgyne* spp. and *R. reniformis* depending on how long the trap crop was grown.

Key words: brown mustard, cover crop, management, oil radish, trap crop.

2.1. Introduction

Root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes are globally important plant-parasitic nematodes, ranking first and seventh, respectively in terms of economic and scientific importance (Jones et al., 2013). These nematodes are especially damaging to agricultural crops in Hawai'i (Sipes, 1994; Sipes and Wang, 2000; Dorman and Nelson, 2012). As conventional nematicides including methyl bromide and fenamiphos became unavailable or use of 1, 3-dichloropropene is highly restricted, cover crops with allelopathic effects are being explored as environmentally sound nematicide alternatives (Wang et al., 2002; Zasada et al., 2009; Hooks et al., 2010). Brassica crops are characterized by production of secondary metabolites, glucosinolates which are degraded by endogenous enzymes called myrosinase to release sulfur-containing bioactive isothiocyanates among other products (Matthiessen and Kirkegaard, 2006). The use of brassica plants to manage soil-borne agricultural pests and pathogens is referred to as biofumigation (Kirkegaard et al., 1993).

Members of Brassicaceae have gained popularity as cover crops because of their soil health improvement services including nutrient scavenging (Kristensen and Thorup-Kristensen, 2004),

biodrilling to improve soil tilth (Chen and Weil, 2011), water conservation, weed smothering, and habitat improvement for conservation of natural enemies of pests and pathogens (Clark, 2008). However, most brassica crops are susceptible to *Meloidogyne* spp. and *R. reniformis* posing an important management challenge (Edwards and Ploeg, 2014; Rudolph et al., 2015; Fourie et al., 2016). This project investigated conventional trap cropping potential of oil radish (*Raphanus sativus*) and brown mustard (*Brassica juncea*) on *M. incognita* and *R. reniformis*.

An effective trap crop for plant-parasitic nematodes is a plant that allows nematode penetration but supports little or no reproduction, thus referred to as dead-end trap crop (Gardner and Caswell-Chen, 1994). Oil radish and whit mustard (*Sinapis alba*) are good examples of dead-end trap crops used against sugarbeet cyst nematode (*Heterodera schactii*) (Gardner and Caswell-Chen, 1994). In contrast, a conventional trap crop is a highly susceptible plant to a targeted insect pest or pathogen with no dead-end properties. The conventional trap cropping approach is commonly used in entomological studies to attract targeted insect pests away from the main crop (Hokkanen, 1991; Hilje et al., 2001). However, the use of conventional trap cropping strategy against plant-parasitic nematodes is limited because of the risk of unwanted reproduction of targeted nematodes. A number of studies have advocated against the use of cover crops like *R. sativus, B. juncea* and *S. alba* to manage *Meloidogyne* spp. because of the management challenge imposed by their susceptibility to the nematode (Ploeg, 2008; Zasada et al., 2009).

One way to make conventional trap crop worth pursuing for managing plant-parasitic nematodes would be to terminate the trap crop before the targeted nematode completes a life cycle (Melakeberhan et al., 2008). It was hypothesized that using brassica crops that are good hosts of *M. incognita* and *R. reniformis* would be effective as a nematode trap crop than using a

poor host. Specific objectives of this project were to 1) examine susceptibility of brassica cover crops to *M. incognita*, 2) determine best oil radish termination time for effective trap cropping effect, and 3) compare conventional trap cropping effects of brown mustard to oil radish against *Meloidogyne* spp. and *R. reniformis*.

2.2. Materials and Methods

Nematode inocula

Meloidogyne incognita or *R. reniformis* were extracted from pure cultures maintained on 'Orange Pixie' tomato (*Solanum lycopersicum*) or 'Iron & Clay' cowpea (*Vigna unguiculata*), respectively in the Plant Pathology Greenhouse at the University of Hawai'i, Honolulu, HI. Nematode eggs were extracted from roots using a 0.6% sodium hypochlorite solution (Hussey and Barker, 1973) followed by centrifugal sugar flotation method (Jenkins, 1964). Eggs were hatched in Baermann trays at 24°C for 14 days before use (McSorley and Frederick, 1991).

S*usceptibility Experiments*

A greenhouse experiment was conducted at the Magoon Research and Teaching Facility (21°18'24.9"N and 157°48'33.1"W) at the University of Hawai'i to examine susceptibility of commonly grown commercial and cover crop oil radish cultivars to *M. incognita*. The commercial oil radish cultivars included 'April Cross' (Park Seed Co., Greenwood, SC), 'Discovery' (Dave's Garden, El Segundo, CA), 'Oshin' (Kitazawa Seed Co., Oakland, CA), 'Alpine', 'Miyashige' and 'Summer Cross' (Johnny's Seeds, Winslow, ME). Two cover crop oil radish cultivars tested were 'Sodbuster' (Petcher Seeds, Fruitdale, AL) and 'Tillage Radish'

(Best Forage, Hudson, IN). Each cultivar was seeded 2 seeds per 4-L tree pot (Greenhouse Megastore, West Sacramento, CA) filled with sterile sand-soil mix at 1:1 ratio (v/v) and thinned to 1 plant per pot prior to nematode inoculation. 'Orange Pixie' tomato was included as a root-knot nematode susceptible control. Fourteen-day-old oil radish or 42-day-old tomato seedlings were inoculated with 300 second stage juveniles (J2) of *M. incognita* delivered through 3 ml of water per pot at 3 insertion points surrounding each seedling. The experiment was arranged in a completely randomized design (CRD) with 4 replications and terminated 28 days after inoculation. Soil was manually homogenized prior to collecting 250 cm^3 soil subsample per pot for nematode extraction by elutriation (Byrd et al., 1976) and centrifugal flotation method (Jenkins, 1964). Roots were rated on a 0-5 scale root-gall index (RGI) according to Taylor and Sasser (1978). Root peelings (1-2 mm thickness) of oil radish tap roots together with fine roots or the entire tomato roots were subjected to acid fuchsin staining (Byrd et al., 1983) and were examined under a dissecting microscope (Leica Microsystems Company, Wetzlar, Germany) for nematode penetration and development.

A second greenhouse pot experiment was conducted to examine susceptibility of 'Caliente 199' (Siegers Seed Co., Holland, MI) and 'Pacific Gold' (Johnny's Seeds) brown mustard or 'IdaGold' white mustard (*Sinapis alba*; Johnny's Seeds) to *M. incognita*. 'Orange Pixie' tomato was included as root-knot nematode susceptible control. The plants were seeded in 524-ml nursery pots (Greenhouse Megastore, West Sacramento, CA) filled with sand-soil mix and the experiment was arranged in a CRD with 6 replications. Roots of 14-day-old mustard or 42-day-old tomato seedlings were inoculated with 100 J2 of *M. incognita* in 100 ml nematode suspension. At 28 days after inoculation, nematode eggs were extracted from the entire roots using a 0.6% sodium hypochlorite solution (Hussey and Barker, 1973) followed by centrifugal

sugar flotation method (Jenkins, 1964). Nematodes in a 250 cm^3 soil subsample from each pot were extracted using elutriation (Byrd et al., 1976) and centrifugal flotation method (Jenkins, 1964).

A third greenhouse pot experiment was conducted to compare 'Sodbuster' oil radish and 'Caliente 199' brown mustard for their susceptibility to *R. reniformis*. 'Iron Clay' cowpea was included as a reniform nematode susceptible control. Plants were seeded in 524-ml nursery pots filled with the sand-soil mix. Roots of 14-day-old seedlings were inoculated with 100 vermiform stages of *R. reniformis*. The trial was arranged in a CRD with 6 replications and terminated 28 days after inoculation. At termination of the experiment, the nematode eggs from entire roots or vermiform stages from 250 cm^3 soil subsample per pot were extracted in the same way as described above.

Oil Radish Termination Age Experiment

A field trial was conducted at Poamoho Experiment Station (21°32'14.8"N and 158°5'20.3"W) in Waialua, HI to determine time of oil radish termination that best serves as a trap crop. Soil type was described as a Wahiawa Soil Series, Oxisol, Tropeptic Eutrustox clayey, kaolinitic, isohyperthermic with pH of 5-6. The test site has mixed populations of *M. incognita*, *M. javanica* and *R. reniformis*. 'Sodbuster' oil radish was seeded in 1.2 × 5.5 m^2 plots at 22.4 kg seed/ha and allowed to grow for 14, 28, 42 or 56 days. A no-brassica-crop (bare ground) control was included and the experiment was arranged in a randomized complete block design (RCBD) with 4 replications. Oil radish or control plots were irrigated using a sprinkler irrigation system. At termination of the oil radish, above ground tissue was collected from three 0.09 m^2-quadrants randomly selected per plot and oven dried at 105℃ for 72 hours to estimate biomass. Oil radish

plants were soil incorporated to 10-cm soil depth using a hand-held rototiller (American Honda Motor Co., Alpharetta, GA). One week after soil incorporation, 'Field Trip' pumpkin (*Cucurbita moschata*) was seeded at 1 m spacing between seeding holes with 5 seeds per plot, and irrigated using drip irrigation. Pumpkin fruits were harvested 4 months after planting. Five plants per plot were uprooted and rated for RGI based on a 0-10 scale (Netscher and Sikora, 1990). Six soil cores from the top 10-cm soil depth were collected per plot and composited into a sampling bag immediately before cover crop termination and at monthly intervals thereafter. Soil was sieved through a 4-mm^2 mesh screen and a 250 cm^3 was subsampled for nematode extraction by elutriation (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964). *Meloidogyne* spp. and *R. reniformis* were enumerated using an inverted microscope.

Brown Mustard and Oil Radish Trap Cropping Experiments

Three field trials were conducted at the Poamoho Experiment Station to compare trap cropping effects of oil radish and brown mustard against *Meloidogyne* spp. and *R. reniformis*. Trial I was initiated on November 10, 2016 in which 'Sodbuster' oil radish and 'Caliente 199' brown mustard were seeded in 1.2 × 5.5 m^2 plots at 11.2 kg seed/ha. A no-brassica-crop (bare ground) control was included and the experiment was arranged in an RCBD with 4 replications. Brassica crops were drip irrigated and grown for 42 days at which time 6 soil cores from the top 10-cm soil depth were collected per plot and composited into a sampling bag. The soil was then sieved through a 4-mm^2 mesh screen and a 250 cm^3 soil subsample was subjected to nematode extraction and enumeration in the same way as described above. This experiment was repeated two more times (Trial II and Trial III) but only comparing brown mustard to no-brassica-crop control. Trial II was planted on July 20, 2017 for 35 days, and Trial III on December 7, 2017 for

49 days. Termination dates for the brassica crops were partly determined by the biomass generated in the particular growing season.

Statistical Analysis

All data were checked for normality using Proc Univariate in SAS 9.4 (SAS Institute Inc., Cary, NC). Skewed data for nematode abundance were log10 (x + 1) transformed prior to one-way analysis of variance (ANOVA) in SAS. Nematode data in the oil radish termination age experiment were subjected to repeated measures analysis after determining no interaction between treatments and nematode sampling dates. Means were separated using Waller-Duncan *k*-ratio (*k*=100) *t*-test for one-way ANOVA and only true means were presented.

2.3. Results

Susceptibility Experiments

All oil radish cultivars examined did not reduce nematode fecundity (eggs/g root; Fig. 2.1A) and root penetration number (Fig. 2.1B) of *M. incognita* compared to root-knot nematode susceptible tomato ($P > 0.05$). On the other hand, soil population density of *M. incognita* on 'April Cross', 'Summer Cross' and 'Tillage Radish' was significantly lower ($P \leq 0.05$) than that on 'Orange Pixie' tomato (Fig. 2.1C). Only 'Sodbuster' had similar ($P > 0.05$) RGI as 'Orange Pixie' (Fig. 2.1D). On the other hand, fecundity (eggs/g root) of *M. incognita* on brown and white mustard cultivars examined was similar ($P > 0.05$) to that on the tomato (Table 2.1). Only 'Caliente 199' and 'Pacific Gold' brown mustard maintained similar ($P > 0.05$) numbers of vermiform stages of *M. incognita* in the soil as the 'Orange Pixie' tomato (Table 2.1). When

'Sodbuster' oil radish and 'Caliente 199' brown mustard were inoculated with *R. reniformis*, nematode fecundity was not different ($P > 0.05$) on the oil radish but was greater ($P \leq 0.05$) on the brown mustard compared to cowpea (Fig. 2.2A). Although soil population density of *R. reniformis* was not different on any of the crops tested, brown mustard supported numerically similar ($P > 0.05$) number of *R. reniformis* vermiform stages as the cowpea (Fig. 2.2B). These results indicated that 'Sodbuster' oil radish and 'Caliente 199' brown mustard were susceptible to both *M. incognita* and *R. reniformis*, and were used in the subsequent trap cropping experiments in the field.

Oil Radish Termination Age Experiment

Oil radish above-ground biomass produced in 56, 42 and 28 days after planting were 5.13, 3.55 and 2.78 t/ha dry matter, respectively, which were significantly higher than the no-brassica-crop control (Table 2.2; $P \leq 0.05$). Regardless of the termination age, oil radish did not suppress ($P > 0.05$) soil population densities of *Meloidogyne* spp. and *R. reniformis* over the 4 months of pumpkin growth compared to the control (Table 2.2). However, RGI on pumpkin was significantly reduced ($P \leq 0.05$) in the 14- or 28-day treatments (Fig. 2.3A) compared to the no-brassica-crop control. In addition, pumpkin yield was 1.74 folds higher in the 28-day treatment than the control (Fig. 2.3B).

Trap Cropping Field Trials

Brown mustard suppressed ($P \leq 0.05$) soil population densities of *Meloidogyne* spp. in Trials I and II by 60 and 50%, respectively (Table 2.3). A similar trend appeared in Trial III where soil populations of *Meloidogyne* spp. were reduced by 71% compared to no-brassica-crop

control (Table 2.3), though it was not statistically different ($P > 0.05$). In contrast, soil population of *R. reniformis* was not suppressed ($P > 0.05$) in Trials I and II (terminated within 42 days) but was suppressed ($P \leq 0.05$) in Trial III (terminated at 49 days after planting) with a 39% reduction (Table 2.3).

2.4. Discussion

Susceptibility Experiments

All the oil radish cultivars examined were considered as poor host of *M. incognita*, with lower J2 penetrating the roots or extracted from the soil, eggs/g roots, or root-gall index except for 'Sodbuster'. Edwards and Ploeg (2014) also found most oil radish cultivars to be poor hosts of *Meloidogyne* spp. Although root-gall index on 'Sodbuster' oil radish was 1.8 folds lower than that on 'Orange Pixie' tomato, it was statistically similar. Thus, 'Sodbuster' was selected and used in the following trap cropping experiment. On the other hand, greenhouse pot experiment confirmed that both 'Caliente 199' and 'Pacific Gold' brown mustard were very susceptible to *M. incognita*, which were consistent with previous findings (Monfort et al., 2007; Edwards and Ploeg, 2014). Though both 'Caliente 199' and 'Pacific Gold' generated similar biomass in the field, 'Caliente 199' contained higher concentration of total glucosinolate concentration in its tissues (72.05 μmol g^{-1} tissue) than 'Pacific Gold' (57.65 μmol g^{-1} tissue) (Rudolph et al., 2015), making it a better brassica crop for biofumigation. Thus, 'Caliente 199' brown mustard was selected for the subsequent experiment to evaluate its potential as a trap crop of *Meloidogyne* spp. None-the-less, 'Caliente 199' brown mustard and 'Sodbuster' oil radish were identified as good hosts of *R. reniformis*, as susceptible as 'Iron Clay' cowpea. These results suggested that

‘Caliente 199’ and ‘Sodbuster’ can be good candidates as trap crops against plant-parasitic nematodes.

Oil Radish Termination Age Experiment

In an attempt to determine the best time of oil radish termination to achieve nematode trap cropping effect in the field, soil populations of *Meloidogyne* spp. and *R. reniformis* were not suppressed in any of the oil radish age treatments, indicating lack of effective trap cropping by ‘Sodbuster’ oil radish. None-the-less, terminating ‘Sodbuster’ at 14 or 28 days after planting slightly reduced root-gall index on the subsequent pumpkin crop compared to the no-brassica-crop control.

Trap Cropping Field Trials

On the other hand, when using a root-knot nematode susceptible host, ‘Caliente 199’ brown mustard as a trap crop, it consistently reduced soil population density of *Meloidogyne* spp. This supported the hypothesis that using a susceptible host as a trap crop would be more effective than using a nematode poor host. However, trap cropping effect against *R. reniformis* was more challenging. While ‘Sodbuster’ oil radish did not reduce soil population densities of *R. reniformis* regardless of cover cropping time and being a good host of *R. reniformis*, growing ‘Caliente 199’ brown mustard longer than 42 days was able to reduce *R. reniformis* population densities in the soil.

2.5. Conclusion

In conclusion, brown mustard provided a good trap cropping effect against *Meloidogyne* spp. if terminated within 42 days after planting, and against *R. reniformis* if terminated 49 days after planting. Further research is needed to investigate possibility of combining trap cropping effect with time-sensitive biofumigation to suppress plant-parasitic nematodes targeting on their most vulnerable stages.

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Tables

Table 2.1. Fecundity (eggs/g root) and soil population density of *Meloidogyne incognita* on brown (*Brassica juncea*) and white (*Sinapis alba*) mustard cultivars in comparison to susceptible tomato (*Solanum lycopersicum*) in a greenhouse experiment.

Crops	Cultivars	Eggs/g root	Vermiform stages/250 cm^3 soil
Brown mustard	'Caliente 199'	120a[1]	7ab
Brown mustard	'Pacific Gold'	130a	13ab
White mustard	'IdaGold'	40a	2b
Tomato	'Orange Pixie'	433a	74a

[1]Means (n=4) for eggs per gram of root and soil population density of *M. incognita* in columns followed by the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

Table 2.2. Effect of oil radish termination age as a trap crop on soil population densities of *Meloidogyne* spp. and *Rotylenchulus reniformis* in a field experiment.

	Termination age of oil radish (days)				
Parameters	0	14	28	42	56
Meloidogyne spp./250 cm^3 soil	1440a[1]	1431a	931a	498a	818a
R. reniformis/250 cm^3 soil	484a	414a	844a	304a	358a
Oil radish biomass (t/ha)	0c	0.3c	2.78b	3.55b	5.13a

[1]Means (n=16) are average of 4 replications with repeated measures over 4 sampling dates during the pumpkin crop. Means in a row followed by the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

Table 2.3. Trap cropping effects of 'Caliente 199' brown mustard (*Brassica juncea*) and 'Sodbuster' oil radish (*Raphanus sativus*) on soil population densities of *Meloidogyne* spp. and *Rotylenchulus reniformis* in field experiments.

	Trial I (November 2016)[1]			Trial II (July 2017)		Trial III (December 2017)	
Parameters	BG[2]	Mustard	Oil radish	BG	Mustard	BG	Mustard
Meloidogyne spp./250 cm^3 soil	450a[3]	179b	243ab	50a	25b	68a	20a
R. reniformis/250 cm^3 soil	418a	436a	386a	523a	323a	258a	157b
Trap crop biomass (kg/ha)	0	1220	1790	0	2080	0	2860
Termination time from planting (d)		42			35		49

[1]Dates when the oil radish or brown mustard were seeded.

[2]BG = bare ground control.

[3]Means (n=4) in a column followed by the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

Figures

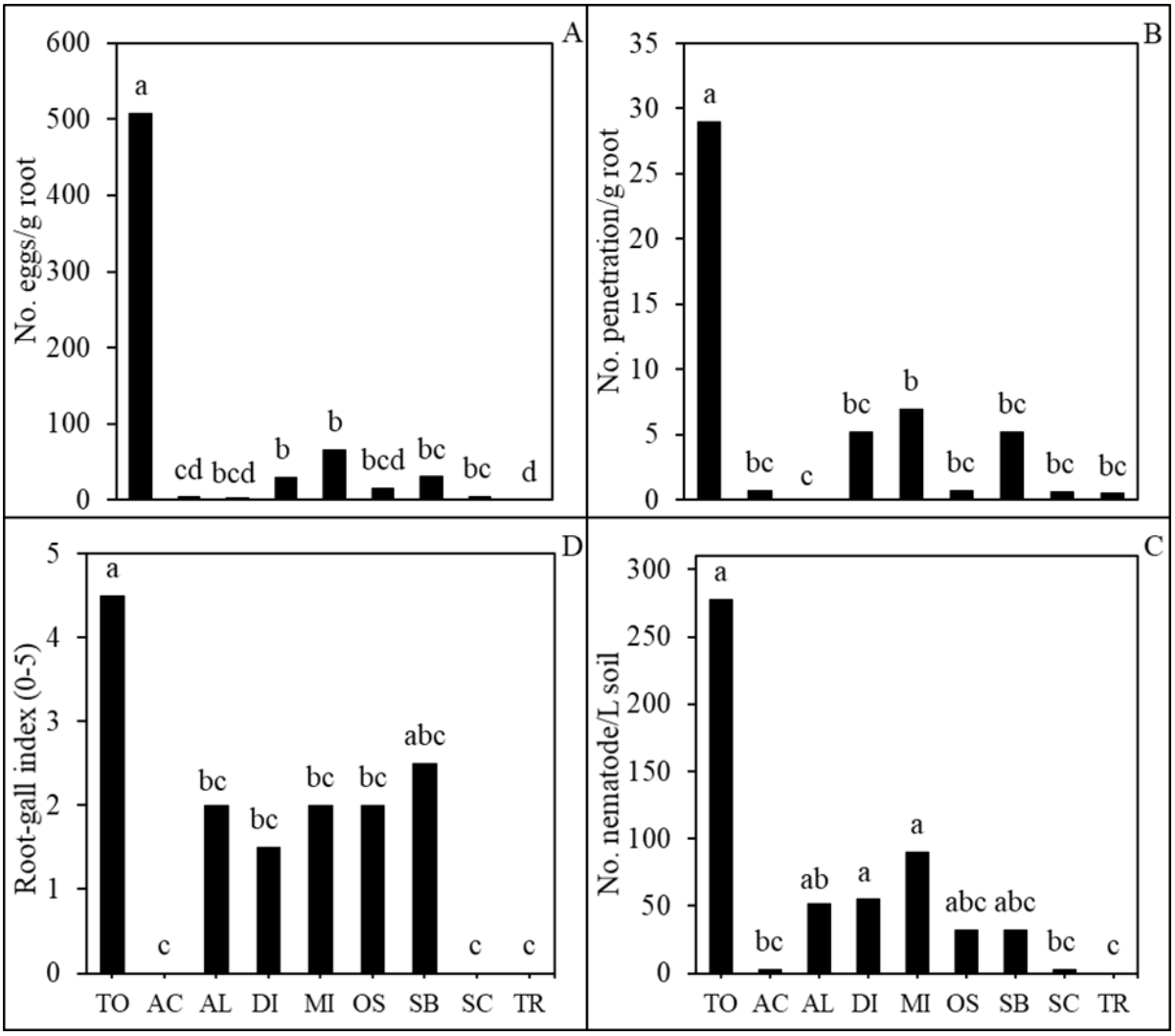

Fig. 2.1 A) fecundity (egg/g root), B) number of nematodes penetrated the roots, C) soil population density of *M. incognita* on oil radish cultivars in comparison to susceptible 'Orange Pixie' tomato in a greenhouse experiment, and D) root galling on tomato induced by *Meloidogyne incognita*. TO=tomato, AC='April Cross', AL='Alpine', DI= 'Discovery', MI='Miyashige', OS='Oshin', SB='Sodbuster', SC='Summer Cross', TR='Tillage Radish'. Bars (n=4) with the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

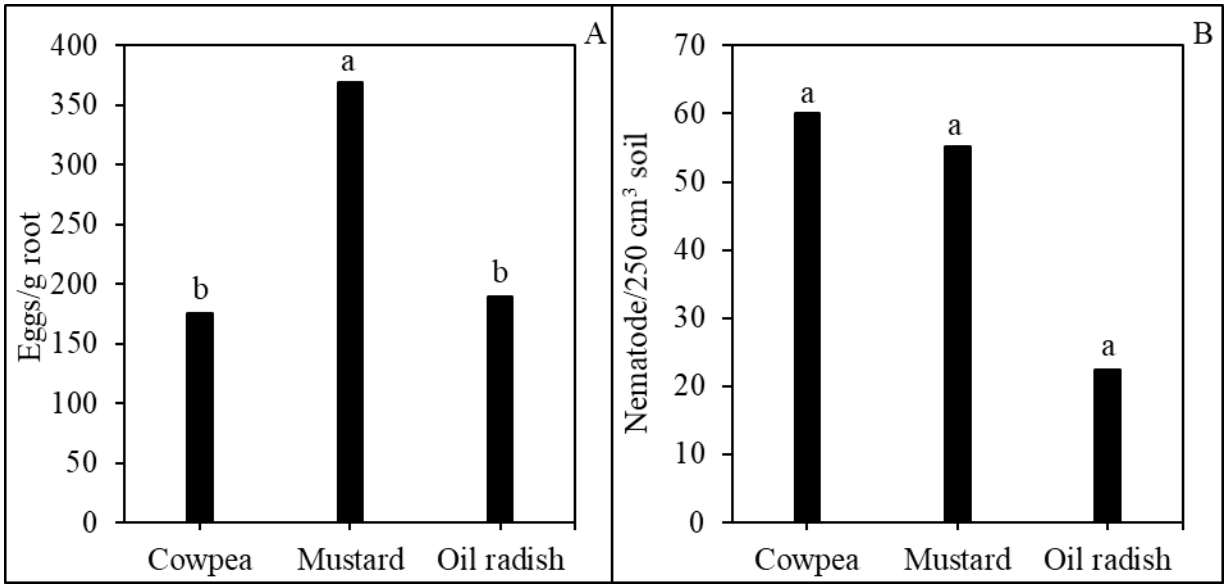

Fig. 2.2. A) Fecundity (eggs/g root) and B) soil population density of *Rotylenchulus reniformis* on 'Caliente 199' brown mustard and 'Sodbuster' oil radish in comparison to susceptible 'Iron Clay' cowpea in a greenhouse experiment. Bars (n=4) with the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

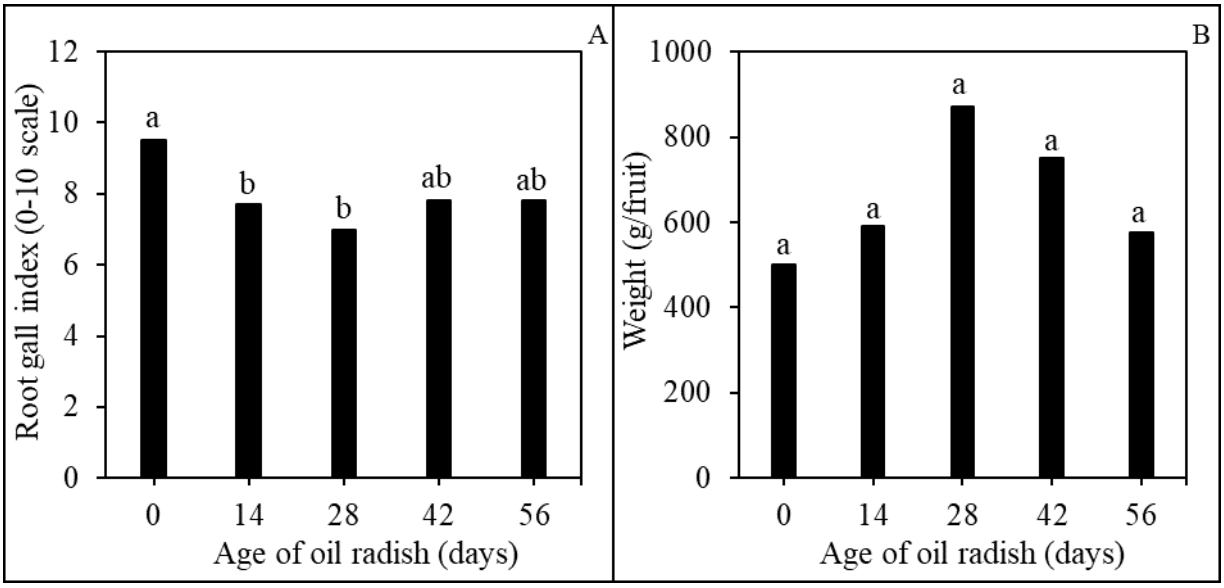

Fig. 2.3. A) *Meloidogyne*-induced root-gall index (0-10 scale) on 'Field Trip' pumpkin, and B) the pumpkin yield (g/fruit) as affected by oil radish termination age field experiment. Bars (n=4) with the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

CHAPTER 3

ENHANCE BIOFUMIGATION OF BRASSICA COVER CROPS FOR PLANT-PARASICTIC NEMATODE MANAGEMENT THROUGH REFINED TERMINATION METHODS

Abstract

Management of plant-parasitic nematodes using biofumigation is always inconsistent. This research aimed to refine biofumigation methods that can consistently enhance its effect on the root-knot (*Meloidogyne incognita* and *M. javanica*) and reniform (*Rotylenchulus reniformis*) nematodes. In greenhouse pot experiments, soil amended with foliage of 'Sodbuster' oil radish (OR; *Raphanus sativus*) suppressed soil population densities of the nematodes compared to a unamended soil control. Three field trials were conducted using OR and 'Caliente 199' brown mustard (MS; *Brassica juncea*) as biofumigant crops in Trial I or only using MS in Trials II and III where the biofumigant crops were subjected to various biofumigation methods compared to a non-biofumigant-crop control. Soil population of *Meloidogyne* spp. were suppressed by OR or MS if the biofumigant crops were macerated (M), tilled (T) into the soil and covered with black plastic (MTBP) in all 3 trials, and reduced zucchini root galls in Trials I and II. However, suppression of *Meloidogyne* spp. was stronger when using MS than OR in the MTBP treatment. Regardless, MTBP also suppressed *R. reniformis* in Trial I but not in Trials II and III. None-the-less, the trend appeared that MTBP reduced *R. reniformis* by 33.9 and 54.9% in Trials II and III, respectively. When soil sulfate and glucose were assayed as indicators of biofumigation, sulfate was more stable in the soil than glucose, ranking in the order of MTBP > MT > T where all of

which were higher than that detected in the biofumigation methods in which brown mustard was terminated by means of no-till and the control. Similar result was obtained when using glucose analysis as indicator of biofumigation when toluene (methylbenzene) was added immediately after sampling to arrest microbial activities but not when toluene was added later. MTBP also stimulated zucchini growth in Trials I and III, but not in II.

Key words: Glucosinolate, glucose, reniform, root-knot nematode, sulfate.**3.1.**

3.1. Introduction

Biofumigation occurs when glucosinolates (GL; β-d-thioglucose thioglycosides) in brassica plant tissues are hydrolyzed by endogenous myrosinase enzyme (Myr; β-thioglucosidase) upon tissue damage to release bioactive isothiocyanates (ITC) among other products such as nitriles, thiocyanates, glucose and sulfate (Kirkegaard et al., 1993; Borek et al., 1994; Fahey et al., 2001). The GL-derived ITC are chemically similar to methyl ITC, the active ingredient in metam sodium, a commonly used fumigant (Matthiessen and Kirkegaard, 2006). Biofumigation is broadly used in management of soil-borne pests and pathogens in cropping systems but its efficacy on plant-parasitic nematodes is inconsistent (Matthiessen and Kirkegaard, 2006). Numerous factors could affect biofumigation efficacy including volatility of ITC (Ntalli and Caboni, 2017), concentration of ITC-producing GL in the biofumigant crops (Gimsing and Kirkegaard, 2006), quality and quantity of brassica tissues incorporated into the soil (Bellostas et al., 2007; Vervoort et al., 2014), soil moisture necessary for GL hydrolysis (Gimsing et al., 2009), soil pH favorable for ITC production (Uda et al., 1986), interference of ITC production by ferrous ions (Hanschen et al., 2015), adsorption of ITC by soil organic matter (Gimsing et al.,

2009), Myr-producing microorganisms in the soil (Ohtsuru et al., 1973; Albaser et al., 2016), vulnerability of the targeted nematode life stage to ITC (Zasada et al., 2009), and methods of biofumigation (Morra and Kirkegaard, 2002). This research focused on refining methods of terminating biofumigant crops to achieve biofumigation efficacy on plant-parasitic nematodes.

A simple biofumigation method in which brassica tissues are incorporated without tissue maceration posts minimal effect on plant-parasitic nematodes (Gruver et al., 2010; Vervoort et al., 2014). Comprehensive maceration of brassica tissues prior to soil incorporation is necessary to increase Myr activity, thus more ITC production and enhancement of biofumigation effect (Morra and Kirkegaard, 2002). Since ITC is prone to volatilization loss, sealing the soil with a roller or covering the soil with a plastic mulch after tissue maceration and soil incorporation can retain ITC in the soil and enhance biofumigation effect (Gimsing and Kirkegaard, 2006).

However, most brassica species and cultivars are good hosts to root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes, two most common plant-parasitic nematodes found in Hawai'i. This has always been a challenge and considered a drawback of using biofumigation to manage these nematodes (Matthiessen and Kirkegaard, 2006; Edwards and Ploeg, 2014). Several literatures suggested that active stages of plant-parasitic nematodes are more sensitive to biofumigation (Mojtahedi et al., 1993; Ploeg, 2008; Zasada et al., 2009). Thus, one way to enhance targeted nematode activity is using susceptible brassica cover crop. This research compared biofumigation effects of less susceptible oil radish (*Raphanus sativus*) vs more susceptible brown mustard (*Brassica juncea*) to *Meloidogyne* spp. (Edwards and Ploeg, 2014). Whereas reports on

the susceptibility of oil radish and brown mustard to *R. reniformis* have been inconsistent (Robinson et al., 1997; Khan, 2005) suggesting variability in cultivars tested, at least our preliminary data showed that 'Sodbuster' oil radish and 'Caliente 199' brown mustard are as susceptible to *R. reniformis* as known susceptible host, cowpea (*Vigna unguiculata*) in a greenhouse pot experiment (Waisen et al., unpublished).

Apart from enhancing nematode activity to be vulnerable to biofumigation, the challenge is the risk of targeted nematodes reproducing and increasing their populations on the susceptible brassica cover crops. This challenge can be addressed by enhancing the ITC release during biofumigation. This research aimed to refine the biofumigation method by integrating tissue maceration, soil incorporation and sealing the soil to enhance the biofumigation effects against the plant-parasitic nematodes.

To determine the efficacy of different biofumigation methods, a direct measurement would be quantifying ITC production using a gas chromatography-mass spectrometry (GC-MS) (Hanschen et al., 2015). However, different detection methods are required for specific ITC compounds produced by different biofumigant crops (Yim et al., 2016). Al-Turki and Dick (2003) used glucose analysis to estimate Myr activities based on increase of glucose content in the soil after biofumigation with relatively reliable results. In this project, besides glucose analysis, we also measure soil sulfate content to compare the effect of different biofumigation methods as sulfate is known to be a byproduct of GL hydrolysis during the ITC production (Borek et al., 1994).

Specific objectives of this research were to 1) screen commercial and cover crop oil radish cultivars for biofumigation effects against *Meloidogyne* spp. and *R. reniformis*; and to compare biofumigation effects of 2) oil radish vs brown mustard, and 3) different biofumigation

(cover crop termination) methods against *Meloidogyne* spp. and *R. reniformis* using glucose vs sulfate as indicators.

3.2. Materials and methods

Screening oil radish cultivars for biofumigation effects

Two greenhouse trials were conducted at Magoon Research and Teaching Facility (21°18'24.9"N and 157°48'33.1" W), University of Hawai'i at Mānoa, Honolulu, HI to examine biofumigation potentials of commercial and cover crop oil radish cultivars against *Meloidogyne* spp. and *R. reniformis*. Commercial cultivars of oil radish examined included 'Alpine', 'Miyashige' and 'Summer Cross' (Johnny's Seeds, Winslow, ME), 'April Cross' (Park Seed Co., Greenwood, SC), 'Discovery' (Dave's Garden, El Segundo, CA), and 'Oshin' (Kitazawa Seed Co., Oakland, CA). Cover crop oil radish cultivars included, 'Sodbuster' (Petcher Seeds, Fruitdale, AL) and 'Tillage Radish' (Best Forage, Hudson, IN). On December 3, 2015, fresh foliage collected from 6-week-old field-grown oil radishes were chopped into 1-2 cm pieces and amended at 1% (dry tissue weight/dry soil weight) into a field soil naturally infested with 2,130 *Meloidogyne* spp. (*M. incognita* and *M. javanica*) and 2,270 *R. reniformis* per 250 cm^3 soil. One-month-old 'Orange Pixie' tomato (*Solanum lycopersicum*) seedlings were transplanted into 15-cm diameter clay pots filled with the soil amended with one of the 8 cultivars of oil radish leaf tissues. A non-biofumigated field soil was included as a bare ground (BG) control and the experiment was arranged in a completely randomized design with 4 replications. The experiment was terminated 1 month after tomato transplanting. Tomato plant height, shoot and root weights, and chlorophyll content were measured at the time of termination. The chlorophyll content was

measured using a hand-held Chlorophyll Meter SPAD-502Plus (Konica, Minolta Sensing Inc., Osaka, Japan). Soil from each pot was emptied into a sampling bag and homogenized by shaking prior to subsampling 250 cm^3 of soil for nematode extraction using elutriation and sugar flotation method (Jenkins, 1964; Byrd et al., 1976).

The above experiment was repeated on April 1, 2016 with the same treatments except that 'Felix' zucchini (*Cucurbita pepo*) was used as nematode bioassay crop instead of tomato. The initial population densities of *Meloidogyne* spp. and *R. reniformis* in this soil were 2,980 and 600 per 250 cm^3 soil, respectively. Same data were collected as described above.

Field trials comparing efficacy of biofumigant crops and biofumigation methods

Trial I

A field trial was conducted at Poamoho Experiment Station, Wahiawa, HI (21°32'14.7"N 158°5'20.2"W) to compare termination methods of brown mustard and oil radish to maximize biofumigation effect against plant-parasitic nematodes. Soil was a Wahiawa Soil Series, Oxisol, Tropeptic Eutrustox clayey, kaolinitic, isohyperthermic with pH of 5-6. The field was naturally infested with 3,400 *Meloidogyne* spp. (mix populations and 760 *R. reniformis* per 250 cm^3 soil as determined on November 17, 2016. 'Sodbuster' oil radish and 'Caliente 199' brown mustard were seeded at a seeding rate of 11.2 kg seeds/ha in 1.2 × 5.5 m^2 plots and drip irrigated. Six weeks later, either oil radish or brown mustard was subjected to various termination methods including 1) no-till (NT) where shoots were clipped off at soil line and the residues covered with a woven weed mat; 2) tissue maceration using a line trimmer followed by soil tillage (MT); and 3) MT followed by covering soil with impermeable black plastic (MTBP). A non-biofumigated bare ground (BG) control was included and the experiment was arranged in

a randomized complete block design (RCBD) with 4 replicated plots. For treatments that involved tillage (T, MT, MTBP and BG), soil was tilled in the top 10-cm depth using a hand-held rototiller (American Honda Motor Co., Alpharetta, GA). Average biomass of oil radish and brown mustard generated was 1.79 t and 1.22 t of dry tissue/ha, respectively, which were equivalent to 0.1% (w/w) amendment rate in the top 10-cm soil. One week after the termination of biofumigant crops, weed mat or impermeable black plastic were uncovered and 2-week-old zucchini seedlings were transplanted at 5 plants per plot and irrigated using drip irrigation. WatchDog® Temperature Data Loggers (Spectrum Technologies Inc., Aurora, IL) were buried 10 cm deep in the soil to record temperatures hourly during the growth of biofumigant crops and removed at 1 week after biofumigant crop termination.

Trial II

This trial was initiated on July 20, 2017 using only 'Caliente 199' brown mustard in the same field as Trial I. Each field plot was again 1.2 × 5.5 m^2. The field was rototilled and the brown mustard was seeded at 11.2 kg seeds/ha in the designated plots. The brown mustard was terminated 5 weeks after planting by 1) no-till (NT) where shoots were clipped off at soil line and residues covered with the woven weed mat; 2) NT followed by tissue maceration using a line trimmer (MNT); 3) MNT followed by covering soil with impermeable black plastic (NTBP); 4) soil tillage without tissue maceration (T); 5) tissue maceration followed by soil tillage (MT); and 6) MT followed by covering soil with impermeable black plastic (MTBP). A no-biofumigant-crop bare ground (BG) control was included and the experiment was arranged in a RCBD with 4 replications. The biofumigant crop tissues in T, MT and MTBP or soil in BG were rototilled into the top 10 cm soil. Average brown mustard biomass generated at termination

was 2.07 t dry tissue/ha or 0.17% (w/w) amendment rate in the top 10 cm soil. One week after biofumigant crop termination, weed mat or impermeable black plastic were uncovered and 2-week-old 'Felix' zucchini seedlings were transplanted. Soil temperature was monitored similar to Trial I. Gravimetric soil moisture was determined right before biofumigant crop termination and 1 week after the termination by oven drying 10-20 g of soil at 70°C for 72 hours.

Trial III

This trial was a technical repeat of Trial II except that the 'Caliente 199' brown mustard was grown for 7 weeks. The trial was initiated on December 7, 2017. Tissues in T, MT and MTBP treatment plots were incorporated into the top 10-cm soil. Average brown mustard biomass generated from this trial was 3.15 t dry tissue/ha which was equivalent to 0.25% (w/w) amendment rate in the top 10 cm soil.

Nematode assay

Six soil cores were collected from top 10 cm of the soil per plot and composited into a sampling bag immediately before termination of biofumigant crops, 1 week after the termination and at monthly intervals over the 3 months of zucchini growth. Soil was sieved through 4-mm^2 mesh screen and homogenized prior to collecting 250 cm^3 soil subsample. Nematodes from the soil subsamples were extracted by elutriation (Byrd et al., 1976) followed by centrifugal sugar flotation method (Jenkins, 1964). Numbers of *Meloidogyne* spp. and *R. reniformis* were counted under a Leica™ Inverted Microscope (Leica Microsystems Company, Wetzlar, Germany).

Soil glucose analysis

A 30 cm^3 of soil subsample was randomly drawn from a composite of 6 soil cores per plot collected immediately before biofumigant crop termination and 1 week after the termination. The soil subsamples were transferred into 60-ml MarketPro® Translucent Soufflé Cups (PJP Marketplace, Philadelphia, PA) and placed on a dry ice in Trial I and Trial III to arrest microbial degradation of glucose. However, in Trial II, soil subsamples were transferred into 50-ml Falcon™ Conical Centrifuge Tube (Thermo Fisher Scientific, Waltham, MA) and immediately added with 2 ml of toluene (methylbenzene, DriSolv®, MiliporeSigma, Darmstadt, Germany) to deactivate microbial activities prior to transportation to the laboratory. At the laboratory, all soil samples were stored in -80°C freezer until use. A subsample of 1 g soil (dry weight equivalent) per sample was transferred into a 50-ml Falcon tube and added with a 0.2 ml of toluene into each tube to deactivate microbial activities. Each soil tube was suspended to 3 ml by adding 0.1 M N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer. The TES buffer was adjusted to pH 7 by titration using 1 M sodium hydroxide before use. Thereafter the TES-soil suspension was vortexed for 20 seconds and incubated at 37°C for 4 hours. The suspension was then centrifuged at 8,000 rpm for 10 minutes before supernatant was filtered through 0.45µm pore Dynarex® Syringe Filters (Dynarex Corporation, Orangeburg, NY) and filtrates were collected in 10-ml round glass vials. Total glucose concentration in each soil filtrate was enzymatically determined using a Glucose (HK) Assay Kit (Sigma-Aldrich Chemical Co., St. Louis, MO) in which the soil filtrate and glucose reagent solution (hexokinase enzyme) were mixed at 1:2 (volume/volume) ratio. A subsample each of 300 µl soil filtrate-glucose reagent solution was pipetted into a well in a 96-well microplate and incubated for 20 minutes at 25°C before adding 1 M silver

nitrate solution (0.25% v/v) to terminate the reaction. Based on a color change due to a formation of 6-phosphogluconate, glucose concentration in the soil filtrate was quantified using a Gen5™ Microplate Reader (BioTek Instruments, Winooski, VT) at 340 nm wavelength with reference to a concentration response curve (y = 0.0133x – 0.0466; R^2 = 0.8833).

The Myr activity was estimated by percent change in glucose concentration immediately before biofumigant crop termination and 1 week after the termination calculated by $\%\ \Delta\text{Myr activity} = [\frac{(\text{G}f - \text{G}i)}{\text{G}i}] \times 100$, where G*i* = soil glucose concentration before biofumigant crop termination, and G*f* = soil glucose concentration 1 week after the biofumigant crop termination. Based on a stoichiometry conducted by Palmieri et al. (1987), *1 mole* of *glucose released* from GL hydrolysis is equivalent to *1 mole* of total *GL hydrolyzed by Myr*.

Soil sulfate assay

Soil samples from each trial were collected immediately before biofumigant crop termination and 1 week after the termination. At each time of sampling, a 5 g of soil subsample from 6 soil cores per plot was randomly drawn into a sterile 50-ml Falcon tube. Then a 25 ml deionized water was added, shook for 30 minutes using a Wrist Action® Shaker (Burrell Scientific LLC, Pittsburgh, PA) and filtered through 2.5-µm Whatman® 42 Filter Paper (Sigma-Aldrich Chemical Co., St. Louis, MO). Extractable sulfate in the soil filtrate was quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Agricultural Diagnostic Services Center at the University of Hawai'i, Honolulu, HI.

Zucchini yield, plant growth and root-gall index

Zucchini fruits were harvested weekly and the plant canopy width and chlorophyll content were measured monthly from 3 zucchini plants per plot. At termination of zucchini crop, 5 plants per plot were uprooted, weighed and rated for root galls caused by *Meloidogyne* spp. based on a 0-10 scale root-gall index (RGI) according to Netscher and Sikora (1990).

Statistical analysis

All data from each experiment or field trial were checked for normality using Proc Univariate in SAS Version 9.4 (SAS Institute Inc., Cary, NC). Wherever necessary, nematode abundance data were normalized using log10 (x + 1) prior to one-way analysis of variance (ANOVA) using Proc GLM in SAS. Nematode data from each field trial were subjected to repeated measures ANOVA to detect any significant interaction between treatments and time of sampling. Means were separated using Waller-Duncan *k*-ratio (*k* = 100) *t*-test and only true means were presented.

3.3. Results

Screening oil radish cultivars for biofumigation effects

All cultivars of oil radish examined suppressed *Meloidogyne* spp. ($P \leq 0.05$) compared to the non-biofumigated BG control regardless of the bioassay crops, tomato or zucchini (Fig. 3.1A-B). Among which, 'Sodbuster', the cover crop oil radish resulted in the lowest soil population density of *Meloidogyne* spp. in the tomato bioassay whereas no difference was

observed ($P > 0.05$) among cultivars on the zucchini bioassay. While all oil radish cultivars tested suppressed *R. reniformis* ($P \leq 0.05$) on the tomato (Fig. 3.1C), only 'Discovery' and 'Oshin' did not suppress *R. reniformis* ($P > 0.05$) on the zucchini compared to the control (Fig. 3.1D). All cultivars of oil radish examined increased ($P \leq 0.05$) tomato shoot weight (Fig. 3.2A), plant height (Fig. 3.2B), and chlorophyll content (Fig. 3.2C) but tomato root weight was not different ($P > 0.05$) among treatments (Fig. 3.2D) compared to the control.

Effects of biofumigation methods using oil radish vs brown mustard

Soil population densities of *Meloidogyne* spp. in all three field trials (Tables 3.1-3.3) and *R. reniformis* in Trial I (Table 3.1) were significantly suppressed when brown mustard was terminated by MTBP compared to the no-biofumigant-crop BG control ($P \leq 0.05$). In addition, brown mustard terminated by T also suppressed ($P \leq 0.05$) abundance of *Meloidogyne* spp. consistently (Tables 3.2 and 3.3). However, terminating brown mustard by MT did not reduce abundance of *Meloidogyne* spp. ($P > 0.05$) in all trials (Table 3.1-3.3). All no-till biofumigation methods including NT, MNT and NTBP using oil radish or brown mustard did not suppress *Meloidogyne* spp. (Table 3.3). Not all biofumigation methods suppressed *R. reniformis* except MTBP using brown mustard in Trial I ($P \leq 0.05$, Table 3.1). On the other hand, RGI on zucchini was suppressed ($P \leq 0.05$) in MTBP by oil radish and brown mustard as well as MT by oil radish in Trial I (Table 3.1) or MTBP by brown mustard in Trial II (Table 3.2) compared to the control. All the other biofumigation methods did not suppress RGI despite suppressing soil population of *Meloidogyne* spp.

Soil glucose vs sulfate as indicators of biofumigation

Percent change in Myr activity immediately before biofumigant crop termination and 1 week after the termination was highest in MTBP and T by brown mustard only in Trial II when toluene was added soon after soil sampling (Table 3.2) but not in Trials I and III (Tables 3.1 and 3.3) when toluene was not added until right before glucose analysis. Although there was no statistical difference detected, on average brown mustard (22.9%) had higher percent change in Myr activity compared to oil radish (5.7%).

When soil sulfate was measured in Trial III, MTBP and MT had the highest soil sulfate concentration, significantly higher ($P \leq 0.05$) than all the no-till treatments as well as the BG control (Table 3.3). Even MNT and NTBP had higher soil sulfate than the BG control ($P \leq 0.05$).

3.4. Discussion

Biofumigation effects of oil radish cultivars on plant-parasitic nematodes

Differences in biofumigation efficacy on *Meloidogyne* spp. were detected among the oil radish cultivars tested on the tomato bioassay trial but not on the zucchini bioassay trial. Oil radish is considered a low ITC-generating GL crop. For example, concentration of ITC-generating GL g^{-1} of dry tissue in 'Bento' oil radish only is 27.8 µmol of (Ngala et al., 2014) compared to 58.4 µmol ITC-generating GL g^{-1} of dry tissue in 'Caliente 199' brown mustard (Rudolph et al., 2015). Based on the results on tomato trial, 'Sodbuster' oil radish was selected for the field trials because it suppressed soil population densities of *Meloidogyne* spp. better than some other oil radish varieties tested.

Effects of oil radish vs brown mustard biofumigation

Biofumigation efficacy was achieved on both *Meloidogyne* spp. and *R. reniformis* when terminating 'Caliente 199' brown mustard by MTBP but not with 'Sodbuster' oil radish in Trial I. This supported the theory that biofumigation with higher ITC-generating GL brassica cover crops would suppress plant-parasitic nematodes more effectively. Besides the fact that 'Caliente 199' contained higher GL than oil radish (Rudolph et al., 2015), it is most likely that higher susceptibility of 'Caliente 199' to *Meloidogyne* spp. than oil radish (Waisen, unpublished) would also play a role in making the targeted nematodes more prone to biofumigation. This same theory could also explain why arugula (*Eruca sativa*) which has intermediate concentration of ITC-generating GL (36 µmol g^{-1} dry tissue) (Ngala et al., 2015) but a poor host of root-knot nematode (*Meloidogyne hapla*) (Edwards and Ploeg, 2014) did not suppress *M. hapla* (Riga, 2011) even when its tissues were soil incorporated and compacted with a roller to minimize volatilization loss of ITC. These results suggest that using a poor host of targeted nematodes to conduct biofumigation might not be a good nematode management strategy. This is because when poor hosts are planted, the targeted nematodes might remain in quiescence, eggs not hatch, or stay in anhydrobiotic stage that can allow them to escape the allelopathic effect of biofumigation.

Effects of biofumigation methods on plant-parasitic nematodes

Results from all three field trials confirmed that the biofumigation methods that had soil tillage treatments performed better than without soil tillage indicating tillage effect.

Despite literature suggestion on tissue maceration could enhance biofumigation effect (Morra and Kirkegaard, 2002), the difference between T alone and MTBP in Trials II and III were not significant in terms of *Meloidogyne* spp. and *R. reniformis* suppression and confirmed by Myr activity and soil sulfate concnetrationas as biofumigation indicators. However, MTBP outperformed MT in terms of numerical reduction of plant-parasitic nematodes in all three trials, and outperformed MT in terms of Myr activities in Trial II and soil sulfate concentration in Trial III. One drawback of using line trimer to perform biofumigation in these small-scale field plots is that the macerated tissues were not entirely captured on the designated plot. Some tissue might have escaped during the line trimming process. MTBP and MT might perform better than T alone if a flail mower was used along with a plastic flap to contain the macerated tissues.

None-the-less, MTBP showed promising plant-parasitic nematode suppression in all field trials because ITC are volatile, sealing soil with black plastic for 7 days would capture the volatile in the soil for a longer period of time. In addition, it is rather consistent that the black plastic raised soil temperature by 2°C compared to the untreated control in all field trials. The slight increase in soil temperature did not reach the lethal temperature to heat kill the nematodes, but higher soil temperatures increase volatility of ITC as well as stimulate the hydrolysis of GL and generate more ITC (Ntalli and Caboni, 2017). It has been known that covering soil with plastic can lead to improvement in biofumgation effect (Gimsing and Kirkegaard, 2006). Although covering the soil with black plastic might also increase soil moisture, another edaphic factor that can enhance hydrolysis of GL, but soil moistures were not different among treatments in Trial II and was in fact lower in MTBP than some of the no-till treatments in Trial III. Thus, soil moisture was not a factor that contributed to better biofumigation in these field trials. Future studies can examine if further wetting the soil after termination of biofumigant crops can

improve the biofumigation effect. This is because Matthiessen et al. (2004) found that irrigation with 34 mm of water after tissue maceration increased propenyl ITC production to 100 nmol/g soil.

In addition, each field trial was terminated at a different length of time partly due to unfavorable weather conditions. While suppression of *Meloidogyne* spp. was equally effective in all three trials, brown mustard terminated at 35 days after planting (DAP) in Trial I achieved better *R. reniformis* suppression than when terminated at 42 (Trial II) and 49 DAP (Trial III). There is no clear explanation on why *R. reniformis* was not suppressed, though there was a numerical trend showing reduction in its numbers, in Trials II and III even with the higher brown mustard biomass at 49 (3.15 t/ha) and 42 DAP (2.07 t/ha) than at 35 DAP (1.22 t/ha).

Soil glucose vs sulfate as indicators of biofumigation

Although glucose analysis works well to indicate performance of biofumigation in Trial II, it did not work in Trial I and III when toluene was not added immediately at soil sampling time to arrest further glucose degradation due to microbial activities as has been documented by Turki and Dick (2003). However, soil sulfate appeared to be a good indicator for biofumigation than soil glucose assay because it is stable in the soil, does not easily degrade by soil microorganisms like glucose, and it is not volatile like ITC.

3.5. Conclusion

Among the two brassica cover crops examined, brown mustard was proven to be an effective biofumigant crop against *Meloidogyne* spp. and *R. reniformis*. Terminating

brown mustard by tissue maceration, soil incorporating followed by covering with black plastic (MTBP) for 1 week achieved effective biofumigation against *Meloidogyne* spp. consistently but its effects on *R. reniformis* was not always significant in the field. The MTBP biofumigation method could be improved by using flail mower. Soil sulfate turned out to be a good indicator for biofumigation and is a good tool for future evaluation of biofumigation methods.

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Tables

Table 3.1. Effects if oil radish and brown mustard biofumigation in field Trial I where cover crops were terminated at 35 days after planting.

	Biofumigant crop termination methods						
	Control	Oil radish			Brown mustard		
Parameters	BG[1]	NT	MT	MTBP	NT	MT	MTBP
Nematode							
Root-knot/250 cm^3 soil	502 ± 88a[2]	506 ± 140a	348 ± 70ab	366 ± 103ab	506 ± 123a	377 ± 85ab	182 ± 31b
Reniform/250 cm^3 soil	706 ± 126ab	818 ± 218a	716 ± 107ab	428 ± 103bc	656 ± 138abc	779 ± 150a	334 ± 75c
Zucchini							
Root-gall index (0-10)	7.2 ± 0.4a	6.1 ± 0.6ab	5.7 ± 0.5b	5.4 ± 0.3b	5.9 ± 0.4ab	6.5 ± 0.4ab	5.6 ± 0.4b
Fruit no.	60 ± 15a	75 ± 15a	69 ± 17a	89 ± 16a	70 ± 8a	82 ± 21a	83 ± 4a
Fruit wt (kg)	3.7 ± 0.9a	5.6 ± 1.5a	4.4 ± 1.5a	5.9 ± 1.0a	5.1 ± 1.1a	5.6 ± 1.7a	5.1 ± 0.6a
Canopy (cm)	35.6 ± 2.2c	42.1 ± 2.0b	41.7 ± 2.1b	41.8 ± 2.3b	41.2 ± 2.1b	38.1 ± 2.4c	47.1 ± 2.2a
Chlorophyll (SPAD)	37.1 ± 1.0b	40.5 ± 0.8a	39.2 ± 0.7a	39.8 ± 0.7a	39.6 ± 0.6a	39.2 ± 0.8a	40.5 ± 0.6a
Soil							
ΔMyr activity (%)[3]	4.5 ± 8.8a	2.7 ± 13.6a	7.7 ± 3.7a	6.8 ± 9.2a	32.2 ± 9.8a	6.7 ± 3.9a	29.8 ±13.6a
Soil moisture (%)	36.5cd	37.9ab	37.3a	35.6a	38.6bc	37.8bcd	37.1d
Temperature (°C)[4]	22(22)	22(23)	22(22)	22(24)	22(23)	22(22)	22(24)

[1]BG = bare ground control; NT = clipping shoots at soil line and covering with woven weed mat in no-till; MNT = tissue maceration in no-till; NTBP = tissue maceration in no-till and covering with impermeable black plastic mulch; T = soil tillage without

prior tissue maceration, MT = soil tillage following tissue maceration, MTBP = covering impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]ΔMyr = percent change in glucose concentration from right before biofumigant crop termination to 1 week after the termination.

[4]Value outside parenthesis is temperature recorded during biofumigant crop growth and the value inside is the temperature during 1 week of covering with/without weed mat or black plastic.

Table 3.2. Effects of brown mustard biofumigation in field Trial II where the cover crop was terminated at 42 days after planting.

	Biofumigant crop termination methods						
Parameters	BG[1]	NT	MNT	NTBP	T	MT	MTBP
Nematode							
Root-knot/250 cm^3 soil	52 ± 12a[2]	89 ± 32ab	346 ± 298a	577 ± 510a	51 ± 24bc	78 ± 30ab	46 ± 21c
Reniform/250 cm^3 soil	407 ± 83ab	483 ± 49a	375 ± 41ab	430 ± 56ab	377 ± 62ab	351 ± 77ab	269 ± 31b
Zucchini							
Root-gall index (0-10)	5.3 ± 0.3ab	6.2 ± 0.4a	5.6 ± 0.4ab	5.9 ± 0.5a	4.6 ± 0.4bc	5.7 ± 0.5a	4.1 ± 0.6c
Root wt (g)	35.8 ± 3.2a	42.1 ± 5.6a	46.5 ± 6.1a	47.0 ± 6.6a	41.2 ± 4.7a	46.8 ± 4.6a	38.1 ± 4.5a
Fruit no.	65 ± 10a	59 ± 7a	65 ± 7a	66 ± 5a	68 ± 4a	69 ± 5a	74 ± 3a
Fruit wt (kg)	5.4 ± 1.3a	5.0 ± 1.0a	5.4 ± 0.4a	5.7 ± 1.4a	6.5 ± 1.7a	6.9 ± 1.0a	6.4 ± 1.3a
Canopy (m)	0.7 ± 0.1a	0.6 ± 0.1a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a
Chlorophyll (SPAD)	40.2 ± 0.7a	38.9 ± 0.8a	40.0 ± 0.8a	39.9 ± 0.7a	40.9 ± 0.6a	40.0 ± 0.8a	40.5 ± 0.6a
Soil							
ΔMyr activity (%)[3]	22.3 ± 9.7cd	41.5 ± 4.8abc	5.1 ± 2.7d	32.7 ± 6.9bc	57.3 ± 12.2a	22.7 ± 8.7cd	45.6 ± 4.7ab
Moisture (%)	29.9 ± 0.9a	31.0 ± 0.5a	31.5 ± 0.9a	30.5 ± 0.9a	31.0 ± 0.5a	30.0 ± 0.7a	30.4 ± 0.7a
Temperature (°C)[4]	29(29)	28(28)	29(29)	28(31)	28(29)	29(29)	27(31)

[1]BG = bare ground control; NT = clipping shoots at soil line and covering with woven weed mat in no-till; MNT = tissue maceration in no-till; NTBP = tissue maceration in no-till and covering with impermeable black plastic mulch; T = soil tillage without prior tissue maceration, MT = soil tillage following tissue maceration, MTBP = covering impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]ΔMyr = percent change in glucose concentration from right before biofumigant crop termination to 1 week after the termination.

[4]Value outside parenthesis is temperature recorded during biofumigant crop growth and the value inside is the temperature during 1 week of covering with/without weed mat or black plastic.

Table 3.3. Effects of brown mustard biofumigation in field Trial III where the cover crop was terminated at 49 days after planting.

	Biofumigant crop termination methods						
Parameters	BG[1]	NT	MNT	NTBP	T	MT	MTBP
Nematode							
Root-knot/250 cm^3 soil	70 ± 36ab[2]	50 ± 16ab	35 ± 9ab	68 ± 24a	10 ± 4c	23 ± 12bc	16 ± 10c
Reniform/250 cm^3 soil	596 ± 187a	403 ± 112a	430 ± 139a	474 ± 109a	315 ± 53a	322 ± 102a	296 ± 84a
Zucchini							
Root-gall index (0-10)	1.8 ± 0.3a	1.6 ± 0.2a	1.9 ± 0.3a	1.7 ± 0.2a	0.9 ± 0.2a	1.3 ± 0.3a	1.6 ± 0.2a
Root wt (g)	27.1 ± 3.1a	27.5 ± 3.0a	24.7 ± 2.4ab	27.4 ± 3.7a	19.3 ± 2.7b	23.7 ± 2.5b	33.2 ± 4.4a
Fruit no.	42 ± 16a	41 ± 18a	49 ± 17a	27 ± 7a	28 ± 11a	32 ± 15a	44 ± 16a
Fruit wt (kg)	2.4 ± 1.0a	3.0 ± 1.4a	2.7 ± 1.4a	1.5 ± 0.5a	1.2 ± 0.6a	1.8 ± 0.9a	2.4 ± 0.8a
Canopy (m)	1.1 ± 0.0bc	1.2 ± 0.1ab	1.1 ± 0.1abc	1.2 ± 0.1ab	0.9 ± 0.1d	1.0 ± 0.1cd	1.2 ± 0.1a
Chlorophyll (SPAD)	39.8 ± 2.0bc	44.1 ± 0.7a	43.1 ± 1.0ab	43.4 ± 0.9a	43.3 ± 0.6ab	38.8 ± 1.7c	43.0 ± 1.4ab
Soil							
ΔMyr activity (%)[3]	15.9 ± 4.6a	27.8 ± 18.5a	22.1 ± 7.0a	20.8 ± 4.8a	10.3 ± 1.9a	15.3 ± 3.5a	25.4 ± 7.4a
Sulfate (ppm)[4]	4.1 ± 1.3e	5.7 ± 1.2de	10.3 ± 0.5bc	9.2 ± 1.4cd	13.1 ± 0.9abc	13.5 ± 1.9a	17.0 ± 2.2a
Moisture (%)	36.5 ± 0.5ab	37.9 ± 0.5ab	37.3 ± 0.5ab	35.6 ± 1.6b	38.6 ± 0.3a	37.8 ± 0.2ab	37.1 ± 0.7ab
Temperature (°C)[5]	22(22)	22(23)	22(22)	22(24)	22(22)	22(22)	22(24)

[1]BG = bare ground control, NT = clipping shoots at soil line and covering with woven weed mat in no-till, MNT = tissue maceration in no-till, T = soil tillage without prior tissue maceration, MT = soil tillage following tissue maceration, MTBP = covering impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]ΔMyr = percent change in soil glucose concentration from right before biofumigant crop termination to 1 week after the termination.

[4]Sulfate = soil sulfate concentration (ppm) 1 week after biofumigant crop termination.

[5]Value outside parenthesis is temperature recorded during biofumigant crop growth and the value inside is the temperature during 1 week of covering with/without weed mat or black plastic.

Figures

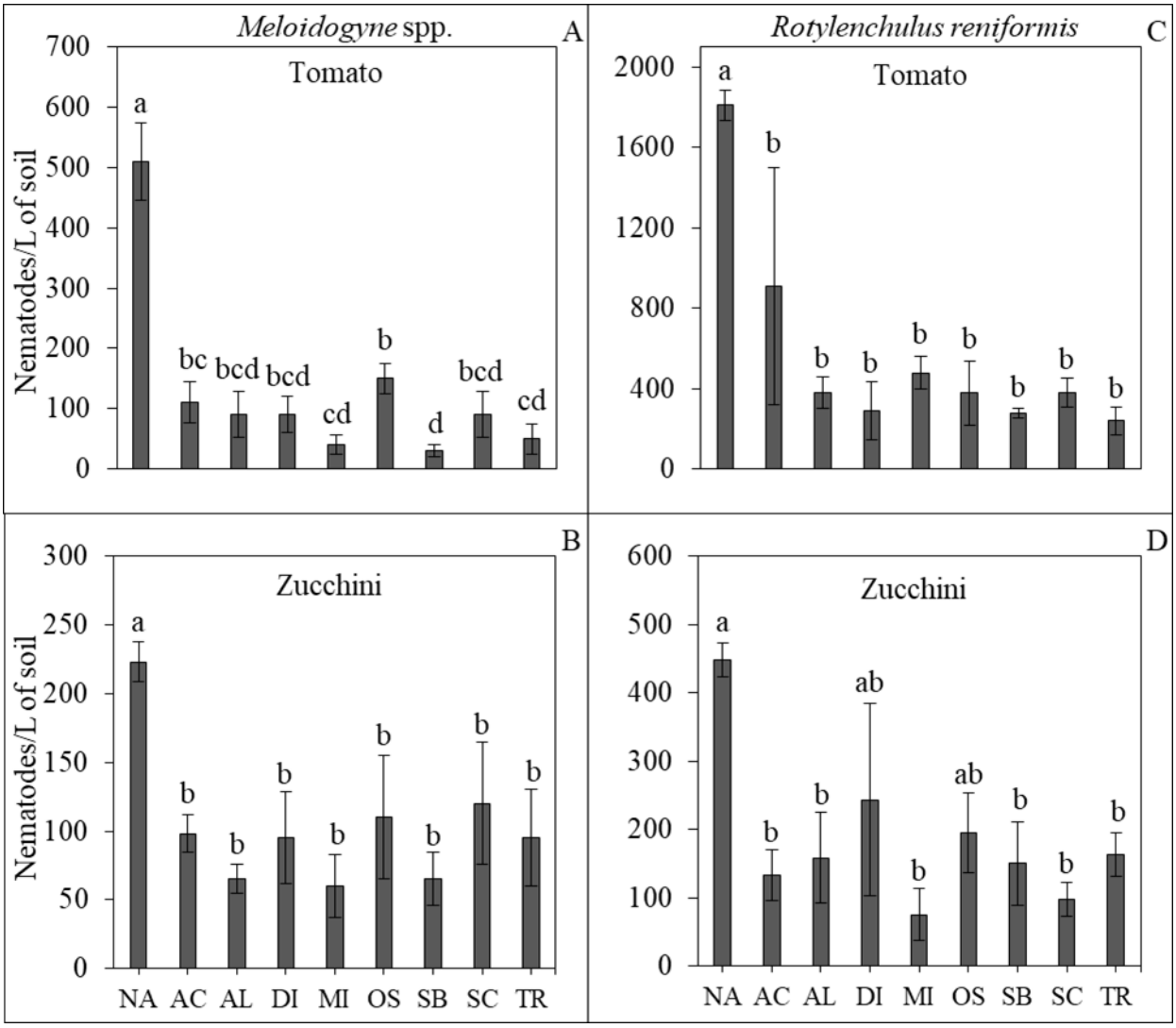

Fig. 3.1. Biofumigation effects of oil radish cultivars, AC = 'April Cross'; AL = 'Alpine'; DI = 'Discovery'; MI = 'Miyashige'; OS = 'Oshin'; SB = 'Sodbuster'; SC = 'Summer Cross'; and TR = 'Tillage Radish' against *Meloidogyne* spp. and *Rotylenchulus reniformis* on tomato 'Orange Pixie' (A-B) or zucchini 'Felix' (C-D) grown in oil radish-amended soil co-infested with the nematodes compared to unamended (NA) control in greenhouse. Bars represent means (n = 4) and those with the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

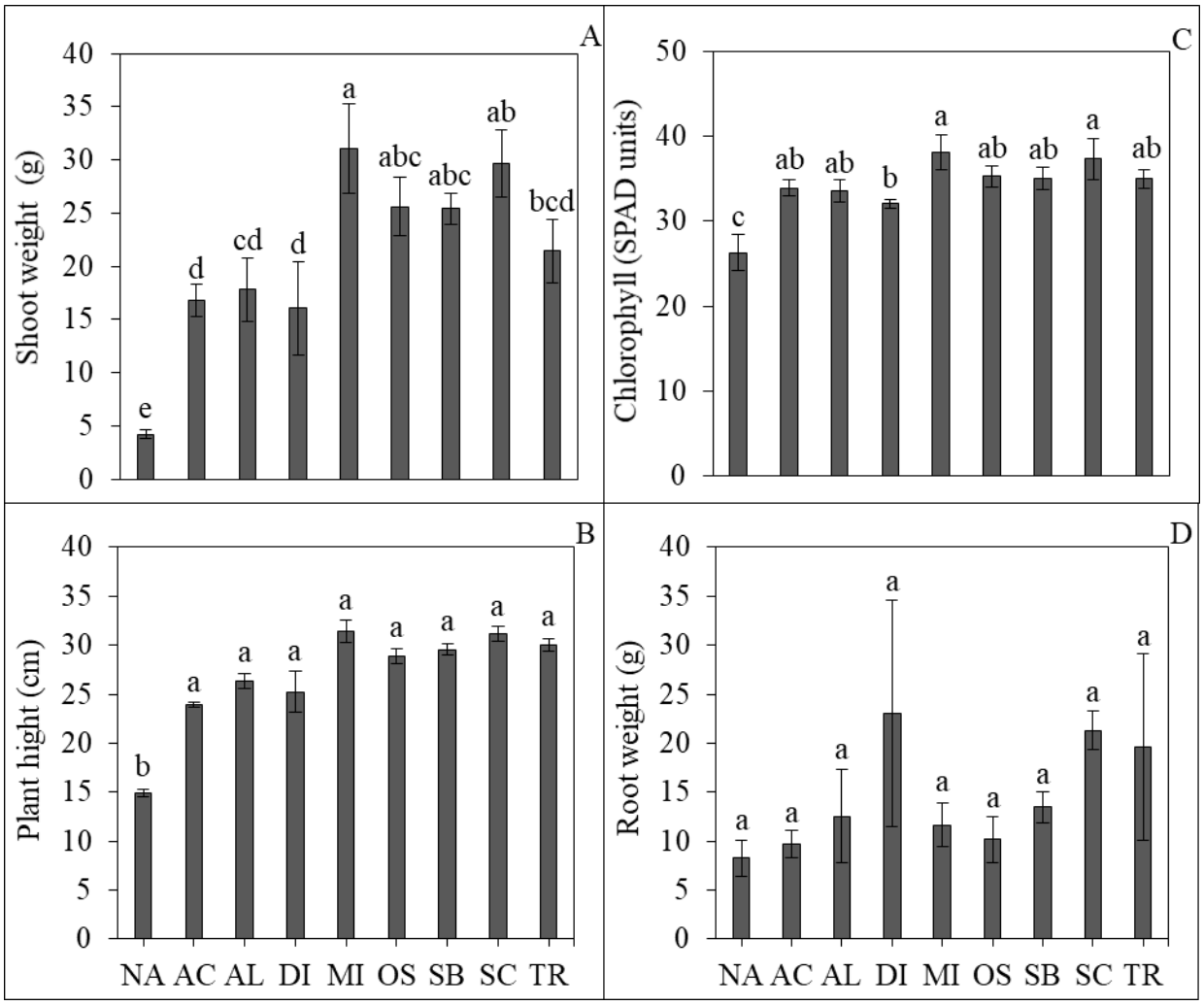

Fig. 3.2. Tomato 'Orange Pixie' Plant growth as affected by biofumigation using oil radish cultivars, AC = 'April Cross'; AL = 'Alpine'; DI = 'Discovery'; MI = 'Miyashige'; OS = 'Oshin'; SB = 'Sodbuster'; SC = 'Summer Cross'; and TR = 'Tillage Radish' against *Meloidogyne* spp. and *Rotylenchulus reniformis* grown in oil radish-amended soil co-infested with the nematodes compared to unamended (NA) control in greenhouse. Bars represent means (n = 4) and those with the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

CHAPTER 4

EFFECTS OF BIOFUMIGATION ON SOIL HEALTH USING NEMATODES AS BIOINDICATORS

Abstract

Limited information is available on the non-target effects of biofumigation on free-living nematodes as indicators of soil health. Objectives of this project were to compare biofumigation effects of oil radish (OR; *Raphanus sativus*) vs brown mustard (MS; *Brassica juncea*) on soil health, determine effects of biofumigation methods with different levels of biofumigation efficacy on free-living nematodes, and examine relationships between biofumigation indicators vs soil health indicators. Three field experiments were conducted in which the biofumigant crops were subjected to various termination methods. Soil samples were collected starting 1 week after biofumigant crop termination and at monthly intervals during zucchini (*Cucurbita pepo*) crop growth. Extracted nematodes were subjected to nematode community analysis. Soil glucose and sulfate analyses were performed on soil samples collected before terminating biofumigant crops and at 7 days after the termination. Both biofumigant crops had no impact on other nematode community indices but instead OR enhanced nutrient enrichment throughout zucchini growth but MS did transiently for up to 1 month after biofumigation. Biofumigant crops terminated by tissue maceration and tillage followed by covering black plastic enhanced soil health indicators but suppressive to plant-parasitic nematodes. Myr activity had a strong positive relationship with soil health indicators when toluene was added in soil samples. However, sulfate was stable in the soil without toluene and even had a stronger positive relationship with the soil health indicators, thus

a good indicator of biofumigation in the field. Effective biofumigation requires soil tillage and may reduce nematodes higher up in the hierarchy of soil food web and their indices.

Key words: Brown mustard, myrosianse activity, oil radish, sulfate, termination methods

4.1. Introduction

Biofumigation is the use of glucosinolate (GL)-derived isothiocyanates (ITC) from brassica plants to manage soil borne pests and pathogens in cropping systems (Kirkegaard et al., 1993). The fact that ITC from brassica are chemically similar to biocidal methyl ITC in metam sodium, there is a likelihood that biofumigation can negatively impact non-target free-living nematodes. Nematodes are ubiquitous and play a major role in soil nutrient cycling (Ferris et al., 2001), and because they are sensitive to variations in land management, correlated with soil functions, useful for explaining environmental processes, comprehensible and useful to land managers, and easy and inexpensive to measure, they are reliable soil health bioindicators (Doran and Parkin, 1994). This research aimed to determine biofumigation effects on free-living nematodes as indicators of soil health.

Several reports on practice of biofumigation did not show negative effects on free-living nematodes and in fact enhance some of the free-living nematodes. Part of the reason is because biofumigant crops also serve as green manure when incorporated into the soil. For example, rototilling white mustard (*Brassica hirta*) followed by irrigation increased free-living nematodes compared to field plots fumigated with metam sodium or 1,3-dichloropropene (Collins et al., 2006). In addition, planting of oil radish (*Raphanus sativus*) and rapeseed (*Brassica napus*) stimulated bacterivorous and fungivorous nematodes,

respectively but did not suppress plant-parasitic nematodes when winter-killed or incorporated by disking and cultipacking (Gruver et al., 2010). Another brassica crop, arugula (*Eruca sativa*), also did not reduce population densities of both free-living nematodes and plant-parasitic nematode (*Meloidogyne hapla*) when terminated by soil incorporation and compacting the soil using a roller (Riga, 2011). Similarly, brown mustard (*Brassica juncea*) did not suppress free-living nematodes and plant-parasitic nematodes (*Trichodorus* spp. and *Tylenchorhynchus* spp.) when tissues were incorporated into the topsoil (Vervoort et al., 2014). All of these literatures suggest that brassica crops are good green manure crops that can increase free-living nematode abundance that can lead to better soil nutrient cycling.

Interestingly, in the literatures related to biofumigation reviewed above, the biofumigation did not suppress the target plant-parasitic nematodes. More evaluation on impact of biofumigation on soil health should be conducted in field trials where the biofumigation is significantly suppressive to the target plant-parasitic nematodes, which could explain why non-target free-living nematodes were not reduced. Also these literatures are evaluating the effects of brassica crops that were terminated by biofumigation methods where tissues were merely incorporated into the soil without intense tissue maceration. For biofumigation to be effective tissues have to be macerated to enhance myrosinase activity and covered with plastic mulch to minimize volatilization loss of ITC.

Another factor that could affect the impact of biofumigation on soil health is the ITC-generating-GL content in the biofumigant crops. It is expected that biofumigation with brown mustard 'Calientee 199' might generated more negative impact on free-living nematodes than oil radish as the former contained 72.05 μmol g^{-1} dry tissue of ITC-GL (Ngala et al., 2015) whereas the later contained 40.7 μmol g^{-1} dry tissue of ITC-GL (Rudolph et al., 2015). In addition, oil

radish is well-known for its ability to alleviate soil compaction through bio-drilling and improve water infiltration (Clark, 2008) due to its swollen root system which has additional soil health benefits than brown mustard.

Therefore, specific objectives of this project were to 1) compare biofumigation effects of oil radish vs brown mustard on soil health, 2) determine effects of biofumigation methods with different levels of biofumigation efficacy on free-living nematodes, and 3) examine relationships between biofumigation indicators vs soil health indicators.

4.2. Materials and methods

Trial I

The first field trial was initiated on November 17, 2016 at Poamoho Experiment Station, Wahiawa, HI (21°32'14.7"N 158°5'20.2"W, 166-215 m elevation). Soil was a Wahiawa Soil Series 1, oxisol order 1, and Tropeptic Eutrustox clayey, kaolinitic, isohyperthermic soil family 1 with pH 5-6. Oil radish 'Sodbuster' (Petcher Seeds, Fruitdale, AL) and brown mustard 'Caliente 199' (Siegers Seed Co., Holland, MI) were seeded at 11.2 kg seeds/ha in 1.2 × 5.5 m^2 plots and irrigated using a drip irrigation. At 42 days after cover crop planting, brown mustard and oil radish were terminated by 1) no-till (NT) where shoots were clipped off at soil line and residues was covered with a woven weed mat; 2) tissue maceration followed by soil tillage (MT); and 3) MT and soil was covered with impermeable black plastic film (MTBP). A no-biofumigant-crop bare ground control was included and the experiment was arranged in a randomized complete block design (RCBD) with 4 replications. Aboveground tissues in MT and MTBP treatment plots were macerated using a line trimmer and roto-tilled to the top 10 cm soil depth using a hand-held

rototiller. Average biomass generated by oil radish and brown mustard were 1.79 t and 1.22 t of dry tissue/ha, respectively, which were equivalent to 0.1% (w/w) amendment rate in the top 10 cm of soil. One week after termination of the biofumigant crops, weed mat and impermeable black plastic mulch were uncovered and 'Felix' zucchini was transplanted 5 plants per plot and drip irrigated. Zucchini fruits were harvested weekly and chlorophyll content measured monthly using a hand-held Chlorophyll Meter SPAD-502Plus (Konica Minolta Sensing Inc., Osaka, Japan). At termination of zucchini crop, 5 plants per plot were uprooted, weighed and rated for root-gall index based on a 0-10 scale according to Netscher and Sikora (1990).

Trial II

Second trial was initiated on July 20, 2017 this time using only 'Caliente 199' brown mustard biofumigant crop and seeded at the same seeding rate as in Trial I. Thirty-five-day old biofumigant crop was subjected to 6 termination methods including 1) no-till (NT) where shoots were clipped off at soil line and residues covered with a woven weed mat; 2) NT but with tissue maceration (MNT); 3) MNT but covered with impermeable black plastic film (NTBP); 4) soil tillage without prior tissue maceration (T); 5) tissue maceration followed by soil tillage (MT); and 6) MT but covered with impermeable black plastic film (MTBP). A no-biofumigant-crop bare ground control was included and the experiment arranged in RCBD with 4 replications. Average biomass generated by brown mustard at termination was 2.07 t dry tissue/ha or 0.17% (w/w) amendment rate in the top 10 cm of soil. One week after the termination of the biofumigant crops, weed mat and impermeable black plastic film were uncovered and 'Felix' zucchini was transplanted at the same planting density as in Trial I.

Trial III

This trial was a technical repeat of Trial II and conducted in the same field as Trial II. The trial was initiated on December 7, 2017 where 'Caliente 199' brown mustard was grown for 7 weeks and tissues in T, MT and MTBP treatment plots were incorporated into the top 10 cm of soil. Average brown mustard biomass generated from this trial was 3.15 t dry tissue/ha which was equivalent to 0.25% (w/w) amendment rate in the top 10 cm soil.

Nematode assay

Soil samples were collected 1 week after biofumigant crop termination and at monthly interval during zucchini growth in each trial. At each sampling time 6 soil cores per plot were collected from the top 10-cm soil depth and composited into a sampling bag. Soil was sieved through 4 mm^2 mesh screen and homogenized by shaking prior to collecting 250 cm^3 soil subsample and nematodes were extracted using elutriation and centrifugal sugar flotation method (Jenkins, 1964; Byrd et al., 1976). All nematodes were identified to genus except for Rhabditidae identified to family level using a Leica™ Inverted Microscope (Leica Microsystems Co, Wetzlar, Germany). Nematode data were subjected to nematode community analysis in which every nematode in a sample was assigned to one of the 5 trophic groups either bacterivores, fungivores, herbivores, omnivores or predators (Yeates et al., 1993) and absolute abundance of each trophic group was enumerated. Nematode richness was determined as the total number of different taxa recorded per sample. Simpson's index of dominance (Simpson, 1949) was calculated using $\lambda = \Sigma (p_i)^2$, where p*i* is the proportion of each of the *i* genera present. Simpson's index of diversity was calculated as $1/\lambda$ (reciprocal of dominance). The fungivore to fungivore and bacterivore ratio (F/F+B) was calculated to characterize decomposition and mineralization

pathways (Freckman and Ettema, 1993). Maturity index (MI) of free-living nematodes defined by Yeates and Bird (1994) was calculated as Σ(p*i* c*i*), where p*i* is the proportion of the taxon, and c*i* is the c-p rating of taxon *i* according to the 1 to 5 c-p scale (Bongers and Bongers, 1998). The nematode fauna was also analyzed by a weighting system of the nematode functional guilds in relation to enrichment and structure of the soil food web (Ferris et al., 2001). The enrichment index (EI) assesses food web responses to available resources, and structure index (SI) reflects the degree of trophic connectance in soil food webs of increasing complexity as the system matures, or progressive food web simplicity as the system degrades (Ferris et al., 2001). These indices were calculated as EI = 100 × [e/(e+b)] and SI = 100 × [s/(s+b)] where e, s, and b are the abundance of nematodes in guilds representing enrichment (guilds Ba1 and Fu2, where Ba1 = guild of bacterivores with c-p 1, Fu2 = fungivores with c-p 2), structure (Ba3-Ba5, Fu3-Fu5, Om3-Om5, Ca2-Ca5, where Om = omnivores, Ca = carnivores = predators), and basal (guilds Ba2 and Fu2) food web components, respectively (Ferris et al., 2001). The channel index (CI) represents the decomposition pathway in the soil food web, was calculated as CI = 100 × [0.8Fu2/(3.2Ba1+ 0.8Fu2)] (Ferris et al., 2001).

Statistical analysis

All data were checked for normality using Proc Univariate in SAS Version 9.4 (SAS Institute Inc., Cary, NC). Wherever necessary data were normalized using log10 (x + 1) or square root transformation prior to analysis of variance (ANOVA) using Proc GLM in the SAS. Nematode data were subjected to repeated measures ANOVA to detect interaction between treatment and date. If significant interaction between treatment and date occurred, data were subjected to one-way ANOVA by date. Means were separated using Waller-Duncan *k*-ratio (*k* =

100) *t*-test and only the true means were presented.

Canonical correspondence analyses (CCA) were performed to detect any relationships between environmental and species variables using CANOCO 4.5 for Windows. Species variables included richness, abundance of nematode trophic groups (bacterivores, fungivores, herbivores, omnivores and predators) including root-knot and reniform nematodes in the soil. Environmental variables included nematode community indices [EI, F/(F+B), CI, MI, SI and richness], indicators of biofumigation (Myr activity and soil sulfate), soil nitrate and temperature, cover crop biomass, zucchini yield (fruit weight), plant growth (chlorophyll content and canopy width) and RGI.

4.3. Results

Effects of biofumigation methods on free-living nematodes

Among the nematode trophic groups present in the test site (Table 4.1), abundance of bacterivorous and fungivorous nematodes were most affected by different cover crop termination methods for biofumigation. No significant interaction was found between treatment and sampling date in Trial I and II, thus repeated measure over the three sampling times were reported in Tables 4.1 and 4.2. Abundance of bacterivores was increased ($P \leq 0.05$) in oil radish terminated by MTBP as well as brown mustard terminated by MT as compared to the no-biofumigant-crop BG control in Trial I (Table 4.2). However, abundance of fungivorous nematodes was reduced ($P \leq 0.05$) in oil radish MTBP or in brown mustard NT and MTBP compared to the BG control in Trial I (Table 4.2). While no significant difference in abundance of bacterivores and fungivores was observed among the six brown mustard termination methods

compared to BG in Trial II (Table 4.3), terminating brown mustard by NT and MT increased omnivorous nematodes ($P \leq 0.05$). This was the only trial where brown mustard termination methods affect abundance of omnivorous nematodes in this study.

In Trial III, significant interaction between treatment and date occurred for bacterivores and herbivores, but not for fungivores, omnivores and predators. Abundance of bacterivores was increased by NTBP, T, MT and MTBP at 1 week after biofumigant crop termination and was increased in all the cover crop treatments ($P \leq 0.05$) except for NTBP at 1 month after the termination compared to the BG control (Table 4.4). However, enhancement of bacterivorous nematodes by brown mustard was no longer apparent ($P > 0.05$) towards the end of the zucchini crop (March 29, 2018). On the other hand, abundance of fungivores were increased by T ($P \leq 0.05$) throughout Trial III (Table 4.4). In all the field trials, abundance of predatory nematodes was sporadic. Although predatory nematodes were increased ($P \leq 0.05$) by oil radish MTBP in Trial I compared to the BG (Table 4.2), the number was very low to be meaningful.

In terms of nematode community indices, effects of biofumigant crop termination was only detected in Trials I and III. In Trial I, responses of nematode community indices were mostly detected in MTBP, either with oil radish or brown mustard (Table 4.2). While MTBP with oil radish significantly increased nematode diversity and EI ($P \leq 0.05$), it reduced F/(F+B), SI, MI and CI ($P \leq 0.05$) in Trial I (Table 4.2). Effects of MTBP by brown mustard was only detected by an increase in diversity and a decrease in SI ($P \leq 0.05$) in Trial I (Table 4.2). In addition, brown mustard MT in Trial I also decreased SI ($P \leq 0.05$).

On the other hand, in Trial III, all brown mustard biofumigation treatments, regardless of till or no till, tissue maceration or no maceration, cover with black plastic or not, increased EI at 7 days after cover crop termination as well as at one month after zucchini planting ($P \leq 0.05$).

Similar to the effects on abundance of bacterivores, this effect on EI dissipated toward the end of the zucchini crop (Table 4.5). Corresponding with the increase in EI by all brown mustard termination methods, CI was decreased in all brown mustard termination methods compared to the BG ($P \leq 0.05$, Table 4.5). All of these treatments except NT reduced MI ($P \leq 0.05$, Table 4.5). However, there was no treatment and time interaction for CI and MI effect. NTBP was the only brown mustard termination methods that increase nematode richness in Trial III ($P \leq 0.05$).

Relationships between biofumigation indicators vs soil health bioindicators

Canonical Correspondence Analyses between species and environmental variables for each trial and their relationships were depicted in ordination diagrams in Fig. 4.1, Fig. 4.2 and Fig. 4.3. The first two canonical axes in ordination diagrams in Fig. 4.1 (Trial I), Fig. 4.2 (Trial II) and Fig. 4.3 (Trial III) explained 88.3, 95.7 or 89.0% of the variance, respectively.

Myr activity was the only biofumigation indicator measured in Trial I and had negative relationship with abundance of root-knot, omnivorous and predatory nematodes, but had positive relationship with EI and soil temperature in Trial I (Fig. 4.1). In Trial II, with a slight modification in the protocol of measuring Myr activity by adding toluene immediately at soil sampling, this biofumigation indicator was negatively related with abundance of herbivores (total plant-parasitic nematode counts), SI as well as chlorophyll content and zucchini fruit weight in Trial II (Fig. 4.2). On the other hand, Myr activity was positively related to abundance of bacterivores, fungivores, predators, omnivores, CI and nematode diversity (Fig. 4.2). Whereas in Trial III, Myr activity was negatively related to RGI, zucchini plant chlorophyll content, fruit weight and soil nitrate concentration at the end of the experiment (Fig. 4.3). On the other hand, a positive relationship was detected between Myr activity with abundance of fungivores and

nematode diversity in Trial III (Fig. 4.3).

In contrast, when using sulfate concentration in the soil as indicator of biofumigation in Trial III, it had a strong negative relationship with CI and MI, but a weaker negative relationship with the abundance of total herbivores, omnivores, reniform and root-knot nematodes, richness and SI (Fig. 4.3). At the same time, sulfate had strong positive relationship with abundance of bacterivores, EI, average soil temperature during the biofumigation period, and biomass of brown mustard (Fig. 4.3).

4.4. Discussion

Effects of oil radish vs brown mustard biofumigation on free-living nematodes

This research found that oil radish and brown mustard did not compromise soil health as indicated by nematode community analysis. Instead, both biofumigant crops examined enhanced the soil health. It is interesting that when comparing soil health conditions based on nematode community analysis in Trial I, NT termination method of either oil radish or brown mustard did not improve soil health in the subsequent zucchini crop. However, terminating these cover crops by MTBP method was the only treatment in this trial that increased nematode diversity, enriched soil nutrients and stimulated more bacterial decomposition than BG. One would anticipate no-till cover cropping system to be more favorable for soil health enhancement. In this case it is not a fair comparison between no-till vs till because this was a newly tilled field site, and only one cropping cycle was going through no-till practice. Soil health condition in MTBP practice reflects a green manure effect with bottom up stimulation of free-living nematodes.

Although no differences in nematode community indices were detected between oil radish and brown mustard terminated by MTBP, stimulation of bacterial decomposition by oil radish MTBP was stronger than brown mustard as reflected on the higher abundance of bacterivores in oil radish than brown mustard MTBP in Trial I. In addition, more soil health indicators were positively affected by oil radish MTBP than that of brown mustard, suggesting that oil radish biofumigation stimulates more nutrient enrichment than brown mustard. The increase in the abundance of bacterivores or reduction in F/(F+B), CI and SI in the oil radish MTBP treatment indicated that bacterial decomposition pathway was enhanced more than fungal decomposition pathway.

Effects of biofumigation methods on free-living nematodes

Results presented here show evaluation of the impacts of biofumigation on soil health was conducted in field trials where the biofumigation was significantly suppressive to the target plant-parasitic nematodes. This is especially true for brown mustard terminated by MTPB. Overall, biofumigation methods evaluated here did not negatively impact abundance of all trophic groups of free-living nematodes except for fungivorous nematodes in Trial I but not in the other trials. The reduction in abundance of fungivores in oil radish and brown mustard MTBP in Trial I could be explained by the high nitrogen content (C: N of ~10) in their shoot biomass (Gruver et al., 2010) that stimulated more bacterial decomposition than fungal decomposition. However, it is unclear why brown mustard terminated by T increased fungivores in Trial III. It is possible that in Trial III, due to weather condition, brown mustard was not terminated until 7 weeks after seeding compared to terminating the cover crops at 5 and 6 weeks after seeding in Trial I and II, respectively. At later stage of growth, C: N ration of brown mustard biomass might

have been changed.

Effects of brown mustard termination methods on nematode community indices were mostly observed on SI or EI. While brown mustard terminated by tillage (MTBP or MT) reduced SI compared to that by no-till in both Trials I and II, all the tillage treatments in Trial III increased EI compared to NT as well as BG at 7 days after termination of the cover crop. However, this effect was short term and did not last till the end of the zucchini crop. Although the decrease in SI by biofumigant crops would suggest a disturbance of soil food web structure, it is most likely due to the increase in bacterivores by the incorporation of these biofumigant crops into the soil that lead to lower SI. Similar trends were observed when terminating oil radish by MTBP. The swollen root system of oil radish was expected to alleviate soil compaction and improve water infiltration (Clark, 2008) and improve soil food web structure over time but not during one cropping of zucchini crop. Although biofumigant crops did not improve soil health thoroughly, they showed significant green manure effects. The bottom line is that, while biofumigation using oil radish and brown mustard was suppressive to target plant-parasitic nematodes, it did not compromise soil health.

Relationships between biofumigation indicators vs soil health indicators

Although each field trial in this study showed slightly different relationships between biofumigation indicators with soil health bioindicators, the relationship between biofumigation indicator with plant-parasitic nematode suppression was rather clear. Biofumigation indicator either Myr activities or soil sulfate concentration was negatively related with one or more of the herbivores indicators (abundance of root-knot, reniform, total herbivorous nematodes, or root-gall index).

In Trial I, although Myr activity showed a strong negative relationship with abundance of *Meloidogyne* spp. and RGI or abundance of omnivores and predators, positive relationship with EI indicated a positive relationship between biofumigation and nutrient enrichment. Higher temperature due to covering the soil with black plastic was related to more myrosinase activities which is expected (Klose et al., 2008). However, lack of significant relationship between Myr activities with many other parameters included in this canonical analysis was most likely due to complication of glucose analysis by microbial activities that degrade the glucose generated from biofumigation (Al-Turki and Dick, 2003). It is possible that storing soil samples in dry ice during transportation from the field to the lab could not effectively arrest microbial activities, thus resulting in a relatively weak relationship between Myr acrivity and other soil health indicators.

In Trial II, toluene was added to the soil sample to deactivate microbial degradation of glucose immediately after soil was sampled in the field. This might have reflected more accurate biofumigation activities that occurred in Trial II. In this trial, Myr activity was negatively related to total abundance of herbivorous nematodes but positively related to abundance of nematodes in all free-living trophic groups. These results were similar to that reported by Al-Turki and Dick (2003). ,. Unlike Trial I, Myr activity in Trial II tended to be negatively related to SI and EI, but strongly related to higher CI, an indication of more fungal decomposition than bacterial decomposition when biofumigation occurred. It is possible that in this trial, the soil health condition in the biofumigated plots had moved towards later stage of the decomposition succession, and were dominated by fungal decomposition pathway.

In Trial III, Myr activity only weakly but negatively related to abundance of plant-

parasitic nematodes, but its negative relationship with RGI was very obvious. In this trial, again toluene was not added immediately after soil sampling inspite of immediately storing the soil samples in dry ice during transportation to the laboratory. Similar to Trial I, fewer significant relationship between soil health indicator and Myr activity were detected in Trial III. Interestingly, soil sulfate, which is also a byproduct of biofumigation during the hydrolysis of glucosinolate (Borek et al., 1994), was negatively related to abundance of all plant-parasitic nematodes as well as nematode richness, abundance of omnivores and SI. The strong positive relationship between sulfate with abundance of bacterivores and predators, EI were very much in line with the results of using Myr activity as indicator of biofumigation in Trial I and II. Due to delay in termination of brown mustard for biofumigation in Trial III (7 week), there is also a strong relationship between sulfate as an indicator of biofumigation with the cover crop biomass, indicating that the more biomass of brown mustard, the stronger the biofumigation effect. Similarly, the higher the soil temperature during biofumigation, the higher the sulfate concentration was detected in the soil.

This is the first report on using soil sulfate to compare biofumigation effect from biofumigant crops. Soil sulfate analysis is a common assay provided by many soil assay laboratories compared to glucose analysis. Using sulfate as an indicator of biofumigation efficiency also does not require special soil treatment to stabilize the product as it is very stable in the soil. Thus, this protocol can be a useful tool for future evaluation on biofumigation studies.

4.5. Conclusion

Biofumigation using either oil radish or brown mustard did not have negative impacts on soil health instead it enhanced soil nutrient cycling through bacteria decomposition and increased nutrient enrichment. Nutrient enrichment as indicated by EI in nematode community analysis was increased throughout a zucchini crop when using oil radish for biofumigation, but brown mustard increased the nutrient enrichment transiently only up to 1 month after the biofumigation. Among the 6 biofumigation methods, MTBP was most effective in enhancing bacterial decomposition. Using glucose analysis to measure Myr activity or soil sulfate as indicator of biofumigation revealed no negative impact of biofumigation on bacterial decomposition, though it occasionally has negative relationship with fungal decomposition.

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Tables

Table 4.1. Nematodes present in the study site grouped into trophic groups, taxa and colonizer-persister values.

Trophic groups	Taxa	Functional guilds
Algaevore	*Achromadora*	al3
Bacterivores	*Acrobeles*	ba2
	Acrobeloides	ba2
	Alaimus	ba4
	Anguilluloides	ba1
	Cephalobus	ba2
	Cervidellus	ba2
	Diplogasteroides	ba1
	Diploscapter	ba1
	Drilocephalobus	ba2
	Eucephalobus	ba2
	Monhystera	ba2
	Panagrellus	ba1
	Panagrolaimus	ba1
	Plectus	ba2
	Prismatolaimus	ba3
	Pseudoacrobeles	ba2
	Pseudocephalobus	ba2
	Rhabditidae	ba1
	Teratocephalus	ba3
	Tylocephalus	ba3
	Wilsonema	ba2
	Zeldia	ba3
Fungivores	*Aphelenchoides*	fu2
	Aphelenchus	fu2
	Filenchus	fu2

	Nothotylenchus	fu2
	Tylenchus	fu2
Herbivores	*Helicotylenchus*	he3
	Meloidogyne	he3
	Paratrichodorus	he4
	Rotylenchulus	he3
	Trichodorus	he4
Omnivores	*Appocelaimellus*	om5
	Appocelaimus	om5
	Dorylaimus	om4
	Eudorylaimus	om4
	Mesodorylaimus	om4
	Pungentus	om4
Predators	*Discolaimus*	ca5
	Mononchus	ca4

Table 4.2. Effects of oil radish and brown mustard termination methods on abundance of nematode trophic groups and indices of nematode community.

		Oil radish			Brown mustard		
Parameters	BG[1]	NT	MT	MTBP	NT	MT	MTBP
Abundance	---------- Nematodes/250 cm3 soil ----------						
Bacterivores	431 ± 108c[2]	469 ± 117bc	511 ± 128bc	598 ± 150ab	342 ± 81c	774 ± 194a	440 ± 110c
Fungivores	257 ± 64ab	340 ± 85b	246 ± 62bc	231 ± 58c	259 ± 65c	526 ± 131a	177 ± 44c
Omnivores	27 ± 7a	36 ± 11a	23 ± 5a	38 ± 8a	49 ± 16a	43 ± 13a	33 ± 6a
Predators	0 ± 0b	0 ± 0b	0 ± 0b	1 ± 1a	0 ± 0b	0 ± 0b	0 ± 0b
Root-knot	502 ± 88a	506 ± 140a	348 ± 70ab	366 ± 103ab	506 ± 123a	377 ± 85ab	182 ± 31b
Reniform	706 ± 126ab	818 ± 218a	716 ± 107ab	428 ± 103bc	656 ± 138abc	779 ± 150a	334 ± 75c
Herbivores	1259 ± 199a	1380 ± 235a	1099 ± 127a	820 ± 130b	1195 ± 169a	1195 ± 207a	564 ± 93b
Indices							
Richness	15 ± 4a	15 ± 4a	15 ± 4a	16 ± 4a	15 ± 4a	17 ± 4a	15 ± 4a
Diversity	5.0 ± 1.2cd	4.1 ± 1.0cd	5.6 ± 1.4bc	6.7 ± 1.7ab	5.0 ± 1.3cd	6.2 ± 1.6abc	15.7 ± 3.9a
EI (%)[3]	58.6 ± 3.2b	58.9 ± 2.9ab	65.3 ± 2.9ab	70.4 ± 3.1a	55.3 ± 4.4b	64.7 ± 3.5ab	65.6 ± 3.1ab
F/(F+B)	0.4 ± 0.1a	0.5 ± 0.1a	0.4 ± 0.1a	0.3 ± 0.1b	0.4 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.1ab
SI (%)	88.7 ± 1.6a	87.7 ± 2.4ab	88.0 ± 2.0ab	82.8 ± 2.8bc	88.3 ± 2.0a	82.0 ± 2.3c	82.9 ± 2.4bc
MI (%)	2.1 ± 0.5a	2.1 ± 0.5ab	2.0 ± 0.5ab	1.8 ± 0.5b	2.1 ± 0.5a	1.9 ± 0.5ab	2.1 ± 0.5a
CI (%)	40.5 ± 5.6a	42.8 ± 4.8a	32.4 ± 6.2ab	20.1 ± 3.8b	45.7 ± 6.9a	34.1 ± 4.4ab	29.6 ± 4.8ab

[1]BG = bare ground control, NT = clipping shoots at soil line and covering with woven weed mat in no-till, MT = soil tillage following tissue maceration, MTBP = covering impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]EI = enrichment index; F/(F+B) = fungivore/fungivore and bacterivore; SI = structure index; MI = maturity index; CI = channel index.

Table 4.3. Effects of brown mustard termination methods on abundance of nematode trophic groups and indices of free-living nematode.

Parameter	BG[1]	NT	MNT	NTBP	T	MT	MTBP
Abundance	---------- *Nematodes/250 cm^3 soil* ----------						
Bacterivore	426 ± 83a[2]	443 ± 78a	422 ± 97a	554 ± 139a	608 ± 101a	731 ± 136a	619 ± 132a
Fungivore	337 ± 67ab	361 ± 69ab	356 ± 94b	303 ± 69ab	533 ± 89a	485 ± 88ab	383 ± 80ab
Omnivore	4 ± 2b	18 ± 4a	4 ± 2b	14 ± 4ab	16 ± 5ab	13 ± 3a	7 ± 2ab
Predator	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	2 ± 1a	0 ± 0a	0 ± 0a
Herbivore	459 ± 79abc	577 ± 67a	726 ± 299ab	1010 ± 516a	396 ± 75bc	433 ± 80abc	319 ± 42c
Root-knot	52 ± 12a	89 ± 32ab	346 ± 298a	577 ± 510a	51 ± 24bc	78 ± 30ab	46 ± 21c
Reniform	407 ± 83ab	483 ± 49a	375 ± 41ab	430 ± 56ab	377 ± 62ab	351 ± 77ab	269 ± 31b
Indices							
Richness	11 ± 1a	13 ± 1a	11 ± 1a	12 ± 1a	13 ± 1a	13 ± 1a	11 ± 1a
Diversity	4.2 ± 0.3a	4.7 ± 0.3a	4.0 ± 0.5a	4.3 ± 0.5a	4.8 ± 0.2a	4.9 ± 0.4a	4.7 ± 0.3a
EI (%)[3]	52.1 ± 3.2ab	54.5 ± 3.1ab	57.7 ± 2.5a	56.7 ± 4.0a	55.9 ± 2.3a	59.0 ± 2.6a	45.4 ± 3.5b
F/(F+B)	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.1a	0.5 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a

SI (%)	10.3 ± 2.5ab	14.8 ± 3.6a	8.0 ± 3.0ab	12.6 ± 3.7ab	12.1 ± 4.6ab	13.7 ± 3.8a	3.6 ± 0.9b
MI (%)	1.9 ± 0.0a	1.9 ± 0.0a	1.9 ± 0.0a	1.9 ± 0.1a	1.9 0 ± 0.0a	1.9 ± 0.0a	1.9 ± 0.0a
CI (%)	52.0 ± 7.3a	48.1 ± 6.5a	39.6 ± 5.0a	48.4 ± 8.9a	45.9 ± 3.3a	57.5 ± 7.5a	37.3 ± 5.1a

[1]BG = bare ground control, NT = clipping shoots at soil line and covering with woven weed mat in no-till, MNT = tissue maceration in no-till, T = soil tillage without prior tissue maceration, MT = soil tillage following tissue maceration, MTBP = covering impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]F/(F+B) = fungivore/fungivore and bacterivore; SI = structure index; MI = maturity index; CI = channel index.

Table 4.4. Effects of treatment-time interactions on abundance of bacterivorous and herbivorous nematodes (numbers/250 cm^3 soil), and enrichment index.

Parameter	BG[1]	NT	MNT	NTBP	T	MT	MTBP
				February 1, 2018			
Bacterivore	108 ± 37d[2]	103 ± 49d	128 ± 43cd	328 ± 171bc	1145 ± 377a	545 ± 153ab	340 ± 122b
Herbivore	133 ± 60a	123 ± 32a	110 ± 50a	230 ± 80a	340 ± 113a	268 ± 184a	58 ± 33a
EI (%)[3]	35.8 ± 5.6d	51.9 ± 3.1c	66.9 ± 1.2b	72.9 ± 4.7ab	84.7 ± 2.5a	79.7 ± 9.2ab	80.3 ± 2.6ab
				February 22, 2018			
Bacterivore	550 ± 135b	1358 ± 612a	1128 ± 198a	798 ± 88ab	1338 ± 175a	1410 ± 375a	1546 ± 273a
Herbivore	313 ± 97a	468 ± 79a	363 ± 35a	505 ± 114a	438 ± 99a	220 ± 78a	263 ± 77a
EI (%)	49.9 ± 5.3b	69.9 ± 6.2a	78.5 ± 1.9a	67.2 ± 7.3ab	75.5 ± 6.1a	76.6 ± 3.0a	74.6 ± 5.1a
				March 29, 2018			
Bacterivore	1810 ± 622a	2420 ± 246a	3768 ± 750a	4698 ± 1508a	2390 ± 710a	2625 ± 957a	3743 ± 1257a
Herbivore	1598 ± 243a	860 ± 283ab	1068 ± 271a	998 ± 209a	305 ± 119b	660 ± 206ab	665 ± 171ab
EI (%)	86.4 ± 4.8a	89.1 ± 3.9a	94.0 ± 1.3a	90.9 ± 3.4a	91.4 ± 1.4a	89.2 ± 1.8a	89.5 ± 2.0a

[1]BG = bare ground control, NT = clipping shoots at soil line and covering with woven weed mat in no-till, MNT = tissue maceration in no-till, T = soil tillage without prior tissue maceration, MT = soil tillage following tissue maceration, MTBP = covering

impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]EI = enrichment index.

Table 4.5. Effects of brown mustard termination methods on abundance of nematode trophic groups and indices of free-living nematode.

[1]BG = bare ground control, NT = clipping shoots at soil line and covering with woven weed mat in no-till, MNT = tissue maceration in no-till, T = soil tillage without prior tissue maceration, MT = soil tillage following tissue maceration, MTBP = covering

Parameter	BG[1]	NT	MNT	NTBP	T	MT	MTBP
Abundance	---------- *Nematodes/250 cm^3 soil* ----------						
Fungivore	350 ± 122b[2]	588 ± 221b	428 ± 132b	441 ± 163ab	582 ± 120a	576 ± 162ab	915 ± 411ab
Omnivore	26 ± 9ab	33 ± 10a	52 ± 19a	45 ± 15a	18 ± 5ab	9 ± 3b	21 ± 7ab
Predator	0 ± 0a	0 ± 0a	3 ± 2a	3 ± 2a	8 ± 6a	2 ± 1a	1 ± 1a
Root-knot	70 ± 36ab	50 ± 16ab	35 ± 9ab	68 ± 24a	10 ± 4c	23 ± 12bc	16 ± 10c
Reniform	596 ± 187a	403 ± 112a	430 ± 139a	474 ± 109a	315 ± 53a	322 ± 102a	296 ± 84a
Indices							
Richness	12 ± 1b	13 ± 1ab	15 ± 2ab	15 ± 1a	14 ± 1ab	13 ± 1b	13 ± 1ab
Diversity	4.1 ± 0.5a	5.1 ± 0.5a	4.8 ± 0.6a	4.7 ± 0.5a	4.1 ± 0.4a	4.2 ± 0.4a	4.9 ± 0.6a
F/(F+B)[3]	0.3 ± 0.1a	0.3 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a
SI (%)	22.6 ± 5.7a	24.0 ± 6.8a	27.0 ± 6.3a	20.5 ± 4.9a	16.3 ± 3.5a	10.5 ± 2.3a	9.1 ± 2.1a
MI (%)	1.8 ± 0.1a	1.7 ± 0.1ab	1.6 ± 0.1bc	1.6 ± 0.1b	1.4 ± 0.0c	1.4 ± 0.1c	1.4 ± 0.1c
CI (%)	44.8 ± 9.8a	19.6 ± 4.7b	12.5 ± 3.3b	16.2 ± 4.5b	11.1 ± 1.5b	13.8 ± 3.7b	12.4 ± 2.5b

impermeable black plastic mulch after tissue maceration and soil tillage.

[2]Means (n = 4) followed by the same letter in a row are not different based on Waller-Duncan *k*-ratio (*k* = 100) t-test.

[3]F/(F+B) = fungivore/fungivore and bacterivore; SI = structure index; MI = maturity index; CI = channel index.

Figures

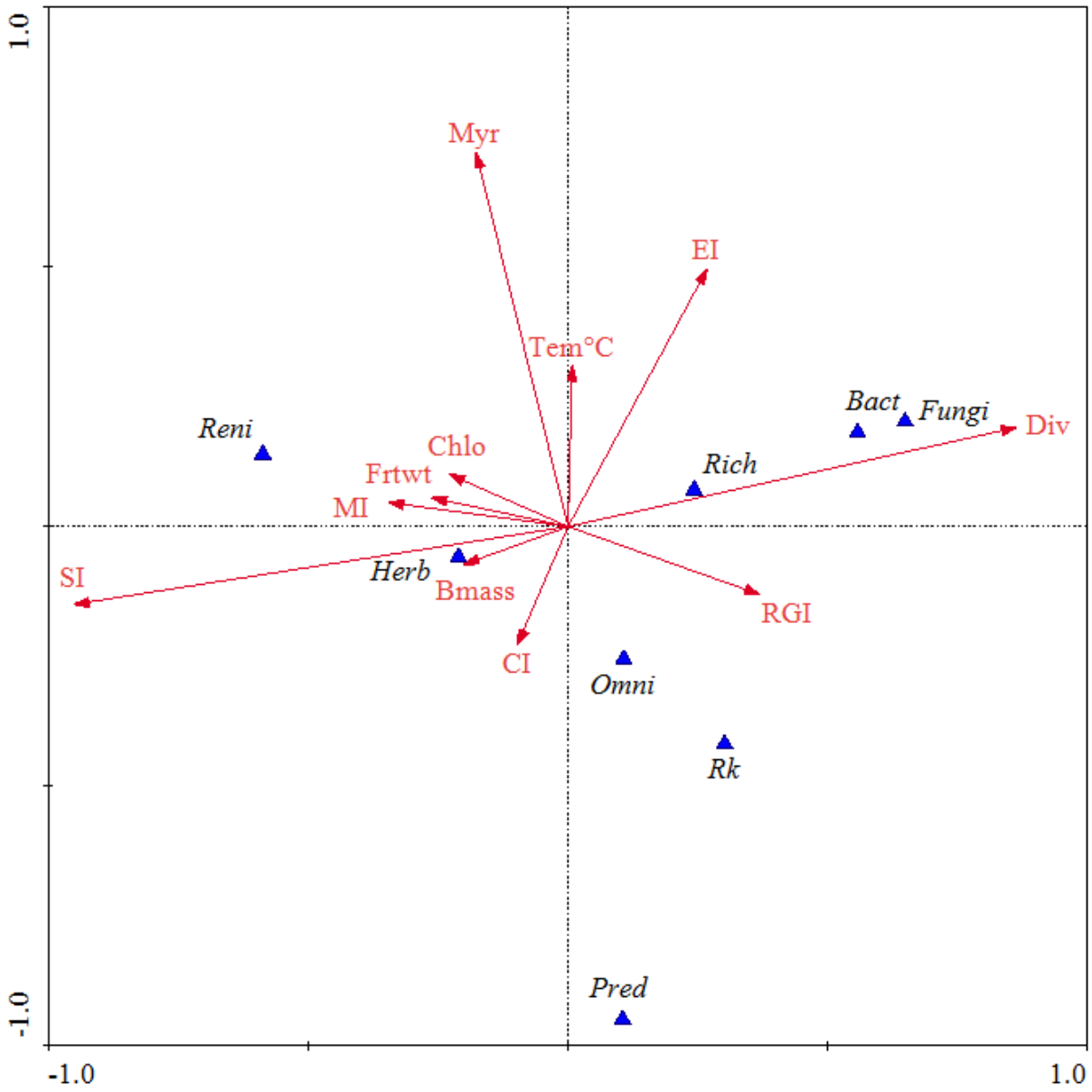

Fig. 4.1. Canonical Correspondence Analyses (CCA) biplot showing the relationships among 8 species variables (blue triangles) and 12 environmnetal variables (red arrows) in zuchini cropping system following oil radish and mustard biofumigation in the field. The species variables include bacterivores (*Bact*), fungivores (*Fungi*), herbovores (*Herb*), omnivores (*Omni*), predators (*Pred*), reniform nematode (*Reni*), richness (*Rich*), and root-knot nematodes (*Rk*).

Environmental variables include biofumigant crop dry biomass (Bmass), zucchini chlorophyll content (Chlo), channel index (C), nematode diversity (Div), nutrient enrichment index (EI), zucchini fruit weight (Frtwt), maturity index (MI), myrosinse activty (Myr), root-gall index (RGI) on zucchini, structure index (SI), and soil temperature (Temp°C). The first two canonical axes explained 88.3% variation of the CCA.

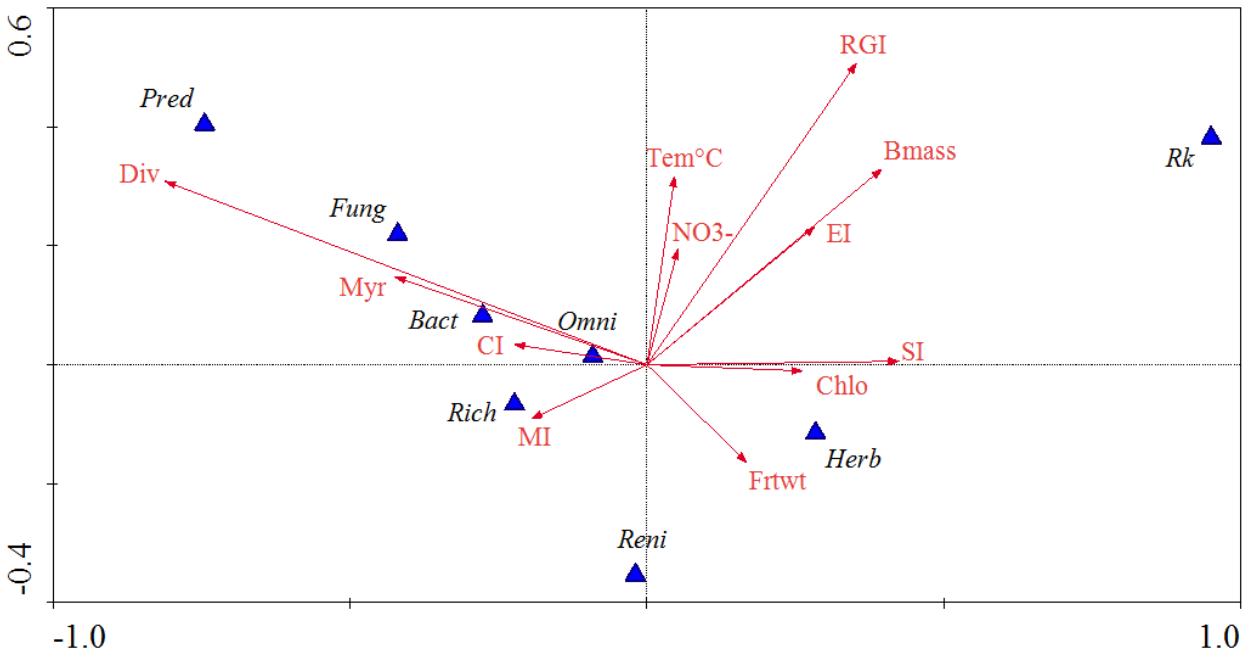

Fig. 4.2. Canonical Correspondence Analyses (CCA) biplot showing the relationships among 8 species variables (blue triangles) and 12 environmnetal variables (red arrows) in zuchini cropping system following brown mustard biofumigation in the field. The species variables include bacterivores (*Bact*), fungivores (*Fungi*), herbovores (*Herb*), omnivores (*Omni*), predators (*Pred*), reniform nematode (*Reni*), richness (*Rich*), and root-knot nematodes (*Rk*). Environmental variables include biofumigant crop dry biomass (Bmass), zucchini chlorophyll content (Chlo), channel index (CI), nematode diversity (Div), nutrient enrichment index (EI), zucchini fruit weight (Frtwt), maturity index (MI), myrosinse activty (Myr), soil nitrate ($NO3^-$), root-gall index (RGI) on zucchini, structure index (SI), and soil temperature (Temp°C). The first two canonical axes explained 95.7% variation of the CCA.

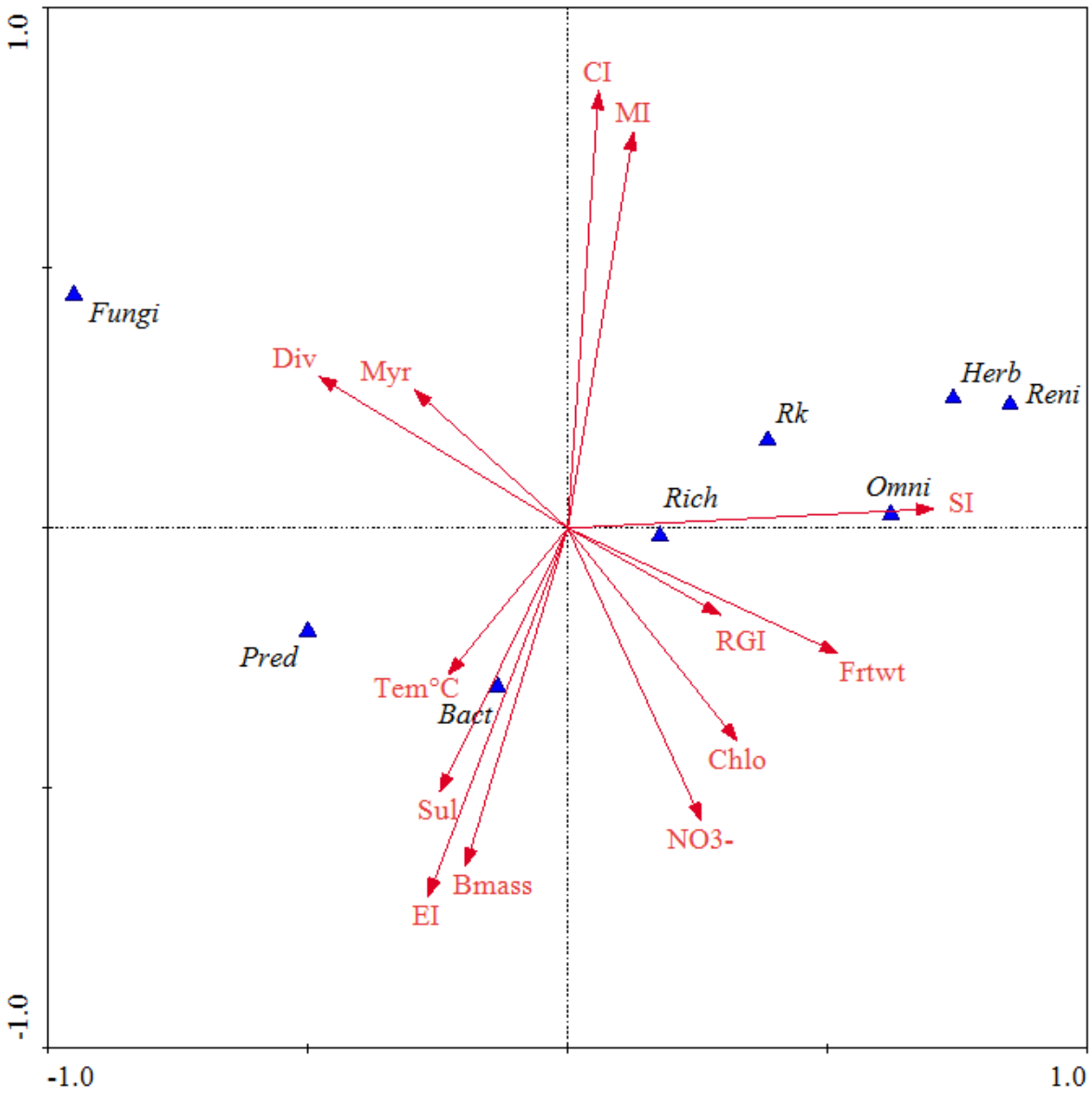

Fig. 4.3. Canonical Correspondence Analyses (CCA) biplot showing the relationships among 8 species variables (blue triangles) and 13 environmnetal variables (red arrows) in zuchini cropping system following brown mustard biofumigation in the field. The species variables include bacterivores (*Bact*), fungivores (*Fungi*), herbovores (*Herb*), omnivores (*Omni*), predators (*Pred*), reniform nematode (*Reni*), richness (*Rich*), and root-knot nematodes (*Rk*). Environmental variables include biofumigant crop dry biomass (Bmass), zucchini chlorophyll content (Chlo), channel index (CI), nematode diversity (Div), nutrient enrichment index (EI),

zucchini fruit weight (Frtwt), maturity index (MI), myrosinse activty (Myr), soil nitrate ($NO3^-$), root-gall index (RGI) on zucchini, structure index (SI), soil sulfate (Sul), and soil temperature (Temp°C). The first two canonical axes explained 89.0% variation of the CCA.

CHAPTER 5

RECOMMENDATIONS AND FUTURE RESEARCH

Based on the mechanism of ITC production and knowledge on the suppressive effects of biofumigation against soil-borne pathogens including plant-parasitic nematodes, previous and current studies have recommended the following steps as necessary to perform effective biofumigation against plant-parasitic nematodes:

1) Select brassicaceous species and cultivars/accessions with high ITC-generating GL (aliphatic and/or aromatic GL) and with high susceptibility to target nematodes.
2) Grow brassica crop in a field infested with target nematodes to stimulate nematode activity since active stages of nematodes are sensitive to biofumigation.
3) Terminate brassica crop before the nematodes reach egg-laying stages to serve as a conventional trap crop. For example, terminate brown mustard 5-6 weeks after planting to trap *Meloidogyne* spp.
4) Acheive sufficient aboveground brassica biomass at least 1.2 metric tons dry biomass per hectare.
5) Macerate/pulverize aerial biofumigant crop tissues with line trimmer/chiper/flail mower to maximize hydrolysis of GL by Myr.
6) Immediately incorporate biofumigant crop tissues thoroughly and evenly within the cultivated soil to maximize contact with nematodes.
7) Add water to achieve 30-37% to enhance GL hydrolysis and to wash ITC to deeper soil profile to get in contact with nematodes.

8) Seal soil with a roller or cover with plastic mulch immediately following tissue incorporation to contain ITC for no more than 7 days.
9) Uncover the plastic and seed/transplant cash crop.

It became clear in the current research that brown mustard is a good biofumigant crop whereas oil radish is a good soil health improvement cover crop. In addition, brown mustard being a good host of *Meloidogyne* spp. would not only stimulate nematode activity rendering them vulnerable to biofumigation but also trap the nematodes if terminated before the nematodes complete a life cycle. Based on these findings, future research could mix both brassica cover crops to enhance biofumigation and improve soil health conditions simultaneously. In a preliminary study, observation on the two brassica plant growth behavior appeared that oil radish smothered brown mustard when seeded along irrigation drip lines. One way to avoid smothering effect could be seeding the brassicas separately on each side of drip lines or broadcast the seeds and irrigate them by sprinkler irrigation.

The fact that sulfur (S) and nitrogen (N) are integral components of GL, their application as fertilizers can increase tissue concentration of GL (Falk et al., 2007; Groenbaek et al., 2016). Fertilizers like ammonium sulfate can be applied at the time of seeding or later not only to increase tissue concentration of GL but also to stimulate plant growth or accumulate sufficient biomass. Fast-release fertilizers should be applied to ensure nutrients are released and be available to the plants. Current study showed that brown mustard biomass production above 1.2 t/ha (dry matter) during 5-6 weeks is critical to control *Meloidogyne* spp.